COE WORKSHOP ON MEMBRANE BIOREACTOR

Microbial Community Analysis in Membrane Bioreactors Treating Municipal Wastewater

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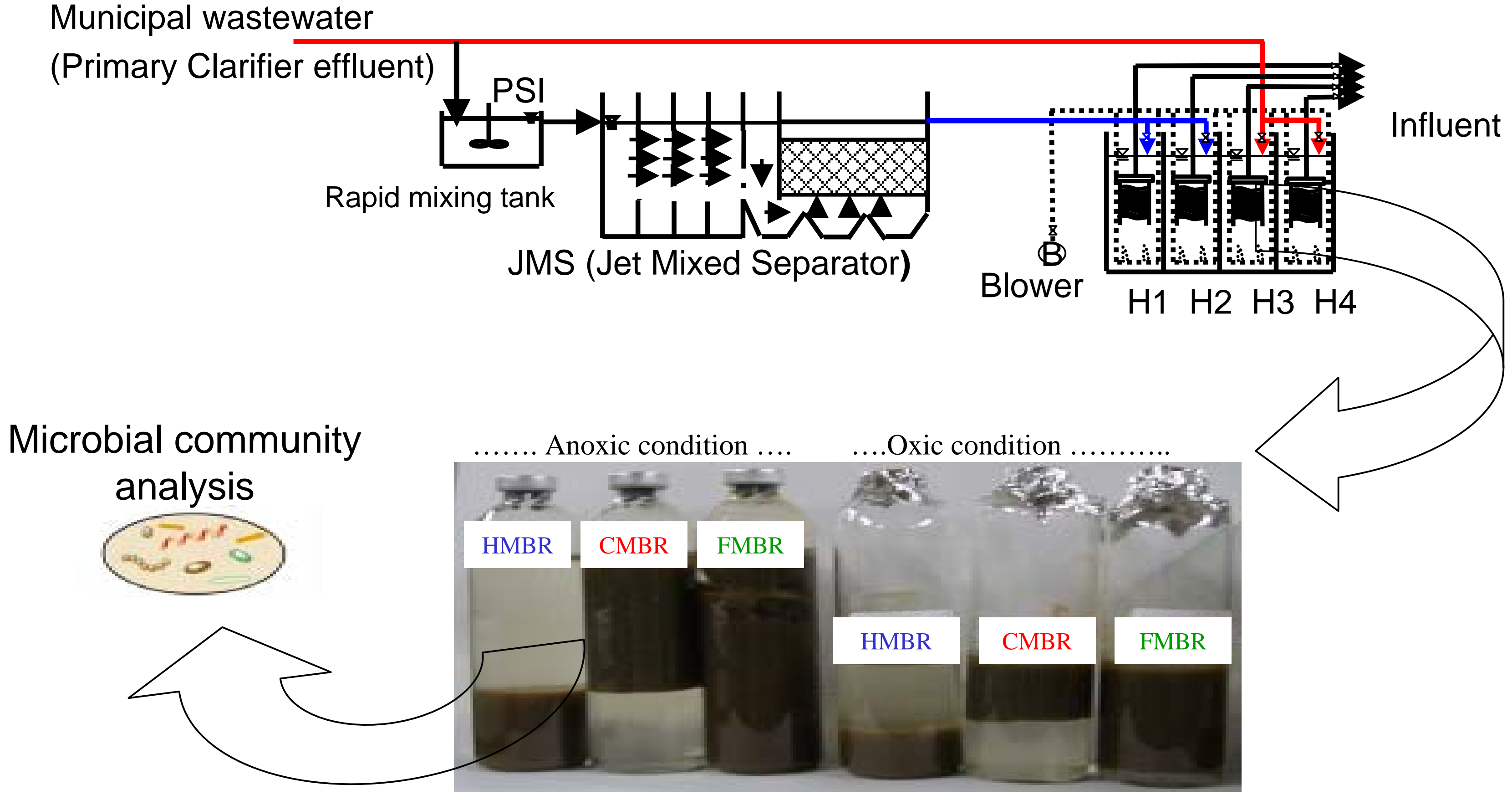
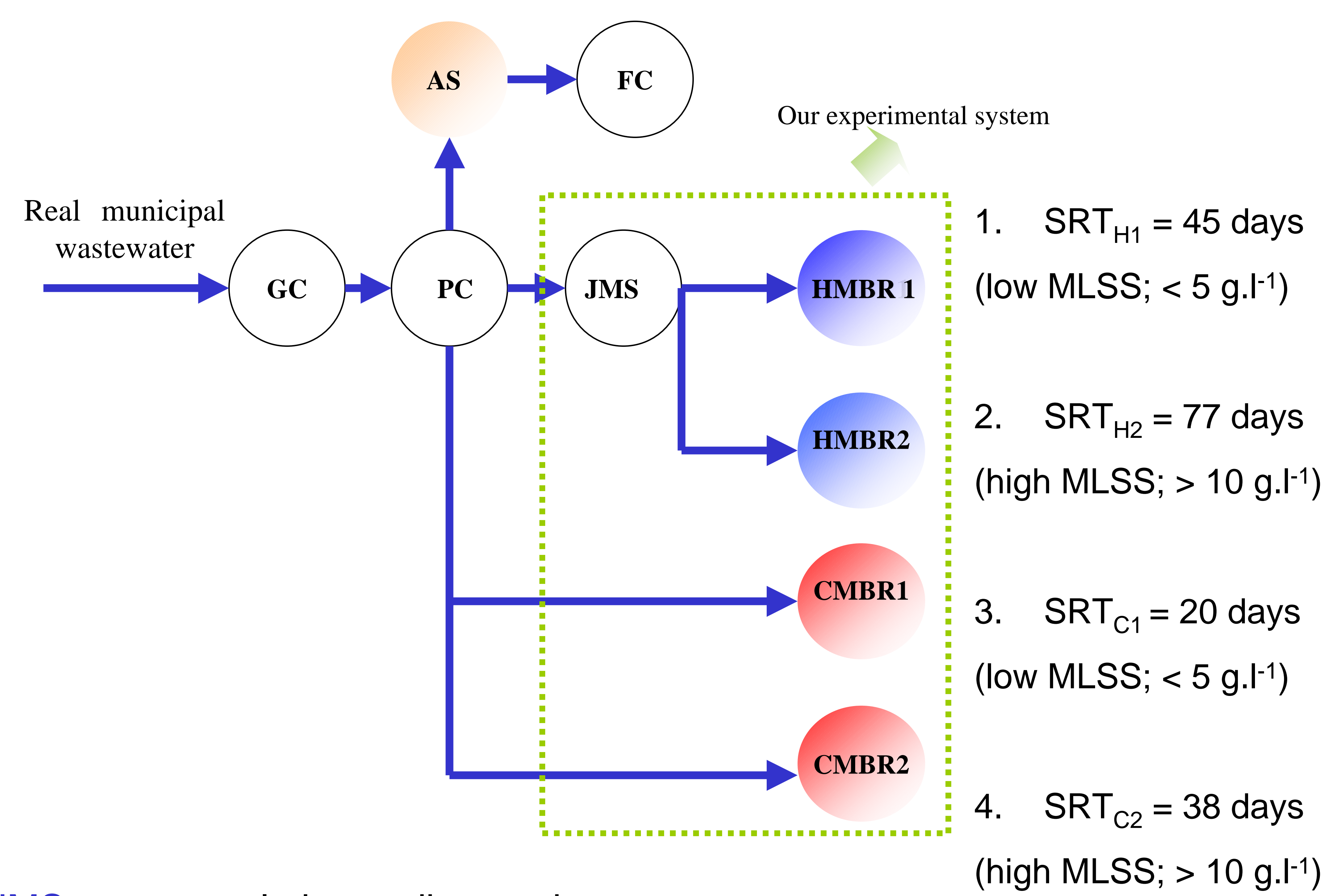
OBJECTIVE



Study and compare the microbial community structure of the recently developed hybrid MBR (HMBR) with the conventional MBR (CMBR) and the most typical activated sludge process and to relate it with the MBR performance

Place site: Municipal wastewater treatment plant, Sapporo city



JMS: pre-coagulation sedimentation process

PART 1

I. Diversity and species distribution in MBRs

II. Microbial community structure in four MBRs comparing with the activated sludge process :

- Detectability of cells by FISH (activity of cells)
- Analysis by PCR-DGGE followed with Dice index of similarity.
- FISH quantitative analysis in a group and subdivision level.

<Mat. & Methods> Flow diagram showing the different steps to study the microbial community < PART 1 >

Microbial community

FISH

Extract DNA

Total community DNA

PCR

Amplify 16S rDNA genes using primers PRBA357F–PRUN518R

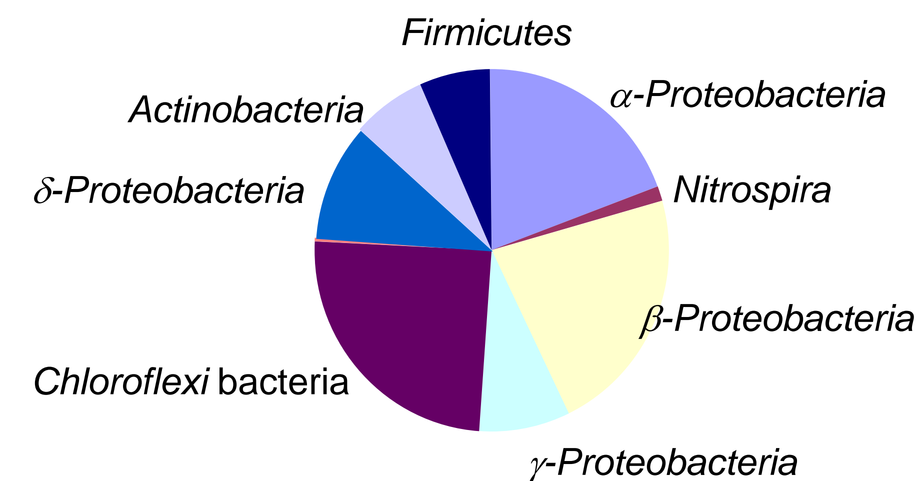
Genetic fingerprints

Month 1 2 3 4 5 6 7 8 9 10

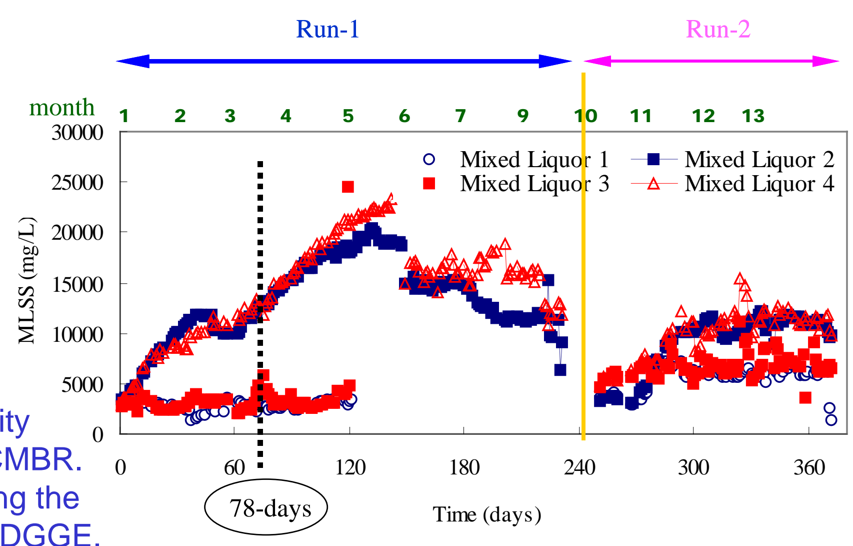
Monitoring the microbial community structure of CMBRs and HMBRs over time and analyzed by Shannon and Equitability index after PCR-DGGE

Bioreactors AS H1 H2 C1 C2

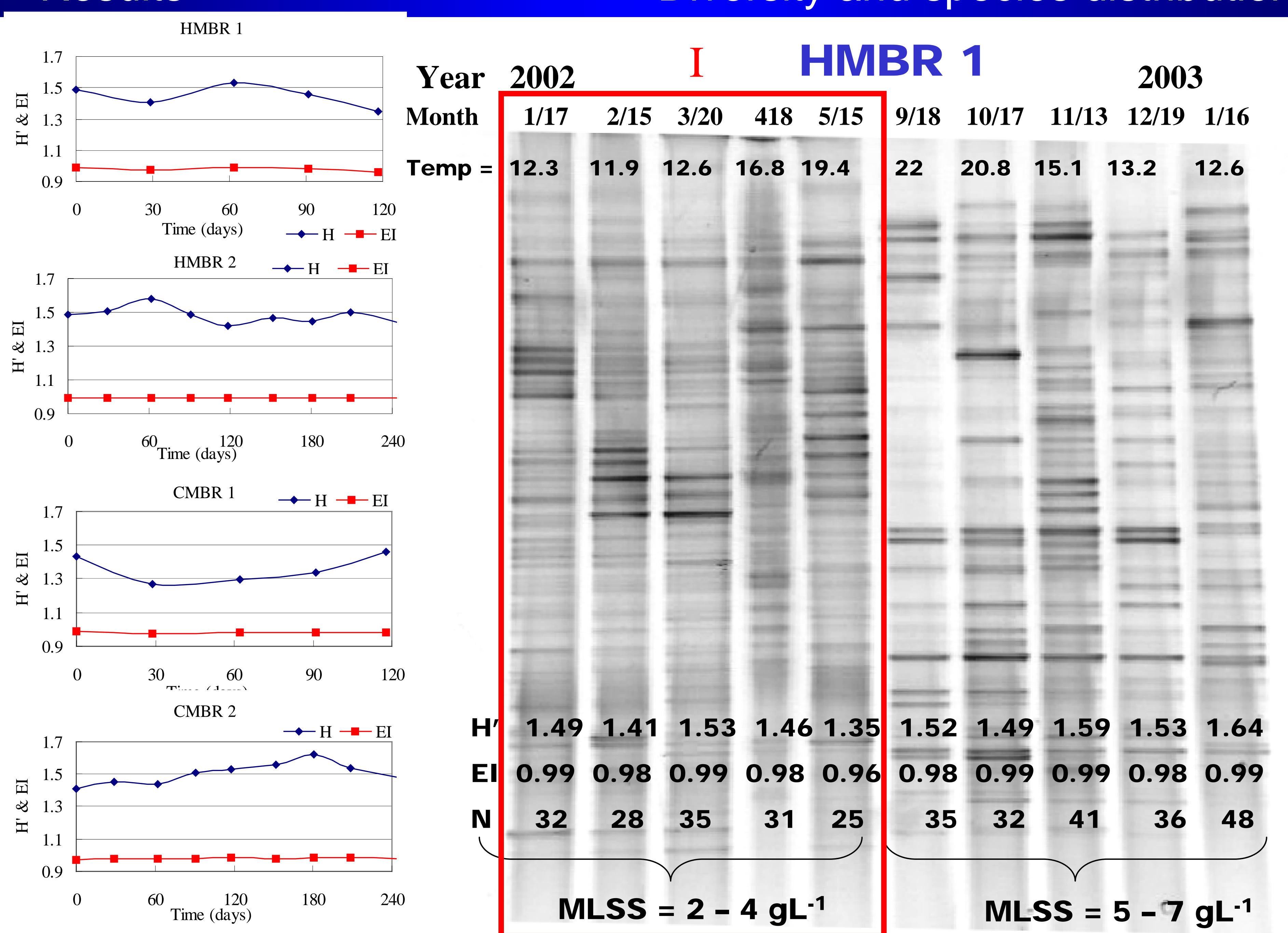
Comparing the microbial community structure for the AS, HMBR and CMBR. The similarities are calculated using the Dice index of similarity after PCR-DGGE.



Microbial community analysis in a group-level for the AS, CMBR and HMBR.



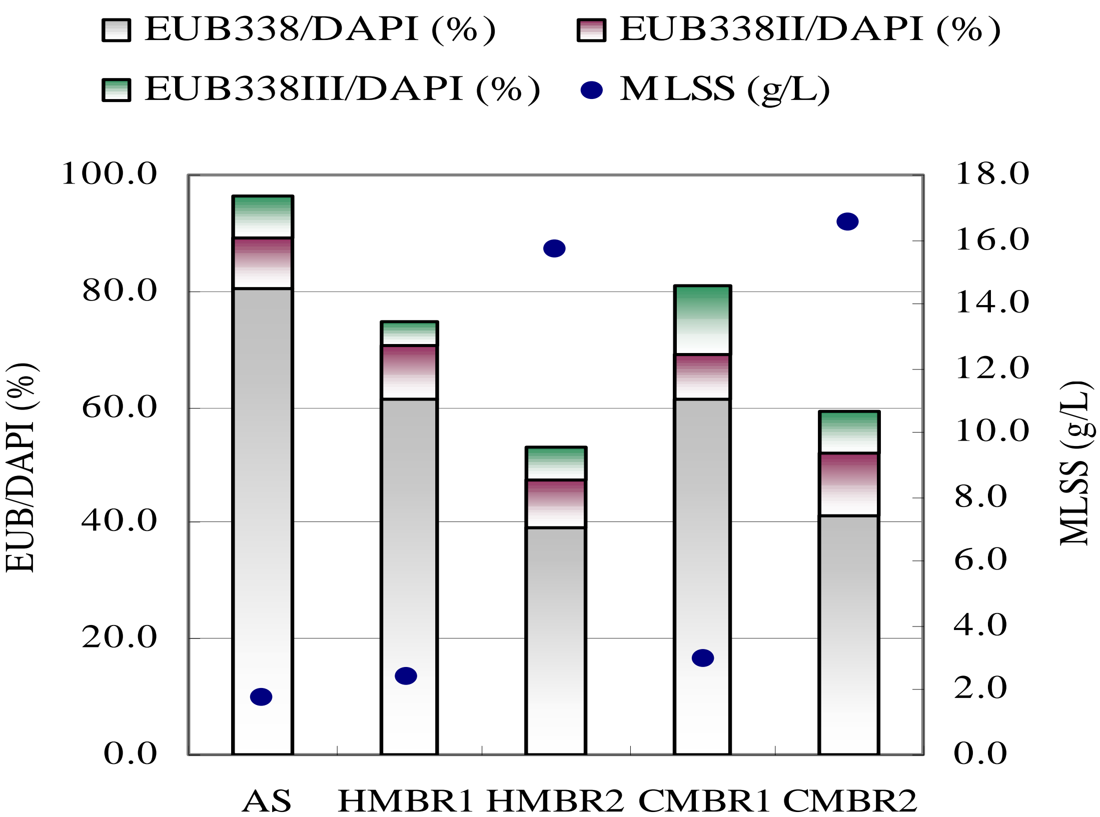
<Results> Diversity and species distribution



Diversity and species distribution

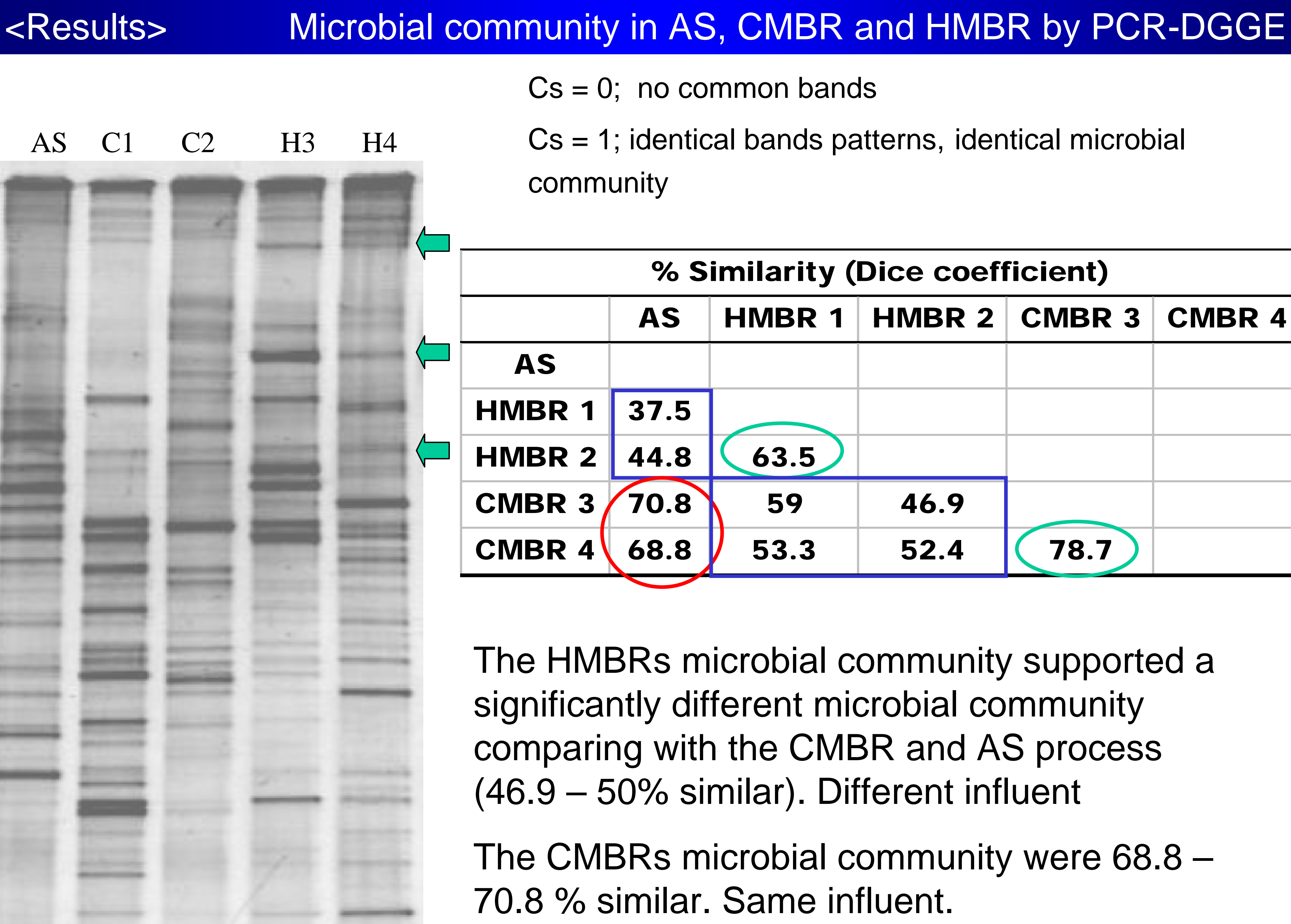
Case study	Primer set	H’	EI	Reference
HMBR 1 treating municipal WW	357-518	1.35-1.64	0.96-0.99	This study
HMBR 2 treating municipal WW	357-518	1.37-1.58	0.99-1.0	This study
CMBR 1 treating municipal WW	357-518	1.27-1.51	0.97-0.99	This study
CMBR 2 treating municipal WW	357-518	1.39-1.62	0.97-0.99	This study
MBR treating graywater (US Navy ship)	332-518	0.82	ND	Stamper *et al*., 2003
Oil contaminated Sakondani Coast (control area)	357-518	1.07-1.16	ND	Ogino *et al*., 2001
Oil contaminated Sakondani Coast (during nutrient application period)	357-518	0.72-0.85	ND	Ogino *et al*., 2001
Oil contaminated Sakondani Coast (after the last nutrient application	357-518	1.18-1.20	ND	Ogino *et al*., 2001
Agricultural soil	338-1491	0.88-1.12	0.73-0.92	Smit *et al*., 2001

<Results> Detectability of cells by FISH in the AS and four different MBRs



The cells in the AS process are very active (>90% have high metabolic activity)

More than 40% of the cells have low metabolic activity (dead, starving or slow growing bacteria) in the HMBR2 and CMBR2. However, the overall activity of microorganisms should be greater in the HMBR2 and CMBR2 due to the high MLSS conc.



% Similarity (Dice coefficient)					
	AS	HMBR 1	HMBR 2	CMBR 3	CMBR 4
AS					
HMBR 1	37.5				
HMBR 2	44.8	63.5			
CMBR 3	70.8	59	46.9		
CMBR 4	68.8	53.3	52.4	78.7	

The HMBRs microbial community supported a significantly different microbial community comparing with the CMBR and AS process (46.9 – 50% similar). Different influent

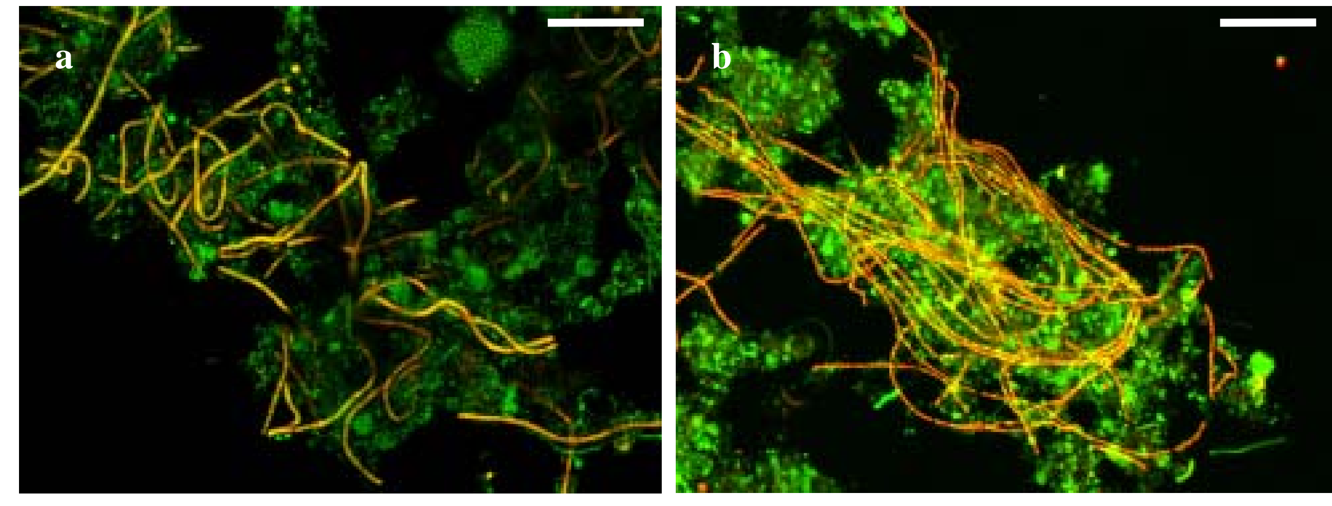
The CMBRs microbial community were 68.8 – 70.8 % similar. Same influent.

Microbial community analysis in a group and subdivision level by FISH

	AS	*HMBR 1*	*HMBR 2*	*CMBR 1*	*CMBR 2*
α- *Proteobacteria*	17.7±15.7	13.3±15.8	11.6±10.2	15.0± 6.6	16.3±14.5
Nitrospira	2.3± 2.6	0	1.5± 3.3	1.0± 0.9	1.2± 1.0
β- *Proteobacteria*	24.7±22.0	21.2±21.6	16.0±18.2	47.0±23.0	23.0±13.0
γ- *Proteobacteria*	9.9±11.0	8.6±11.7	9.2±12.2	12.0± 4.0	9.0±11.0
δ-*Proteobacteria*	5.5± 3.6	1.6± 4.3	4.2± 2.6	6.0± 6.2	10.0± 5.2
Chloroflexi	11.6±12.9	0	5.8± 5.0	0	19.2±21.4
Bacteroidales	1.5± 2.2	0	3.1± 2.7	0.9± 2.0	0.7± 1.2
Actinobacteria	14.1±14.0	7.6±10.0	3.0± 4.3	1.4± 1.6	6.9± 4.6
Firmicutes	2.7± 3.6	0	4.0±10.3	4.2± 5.3	4.7± 7.2
Other bacteria*	10.0	47.7	41.6	12.5	9.0

α- & β-Proteobacteria were the dominant members in all bioreactors.

Chloroflexi bacteria



Activated sludge   CMBR

Chloroflexi bacteria

Molecular phylogenetic surveys indicated that members of the *Chloroflexi* are found in numerous diverse habitats such as activated sludge, geothermal springs, hypersaline mats, deep subsurface, anaerobic/aerobic reactor, anaerobic reactor and dechlorinating enrichments and there appear numerous important roles (degradation of macromolecules, dehalogenation of tetrachloroethene, bulking, etc) of *Chloroflexi* in wastewater treatment plants and these roles have not been well studied in relation to *Chloroflexi* microbial ecology.

Abundant but little known –with few culture representatives- group of bacteria

<Hypothesis 1> *Chloroflexi* bacteria are related with CMBR performance

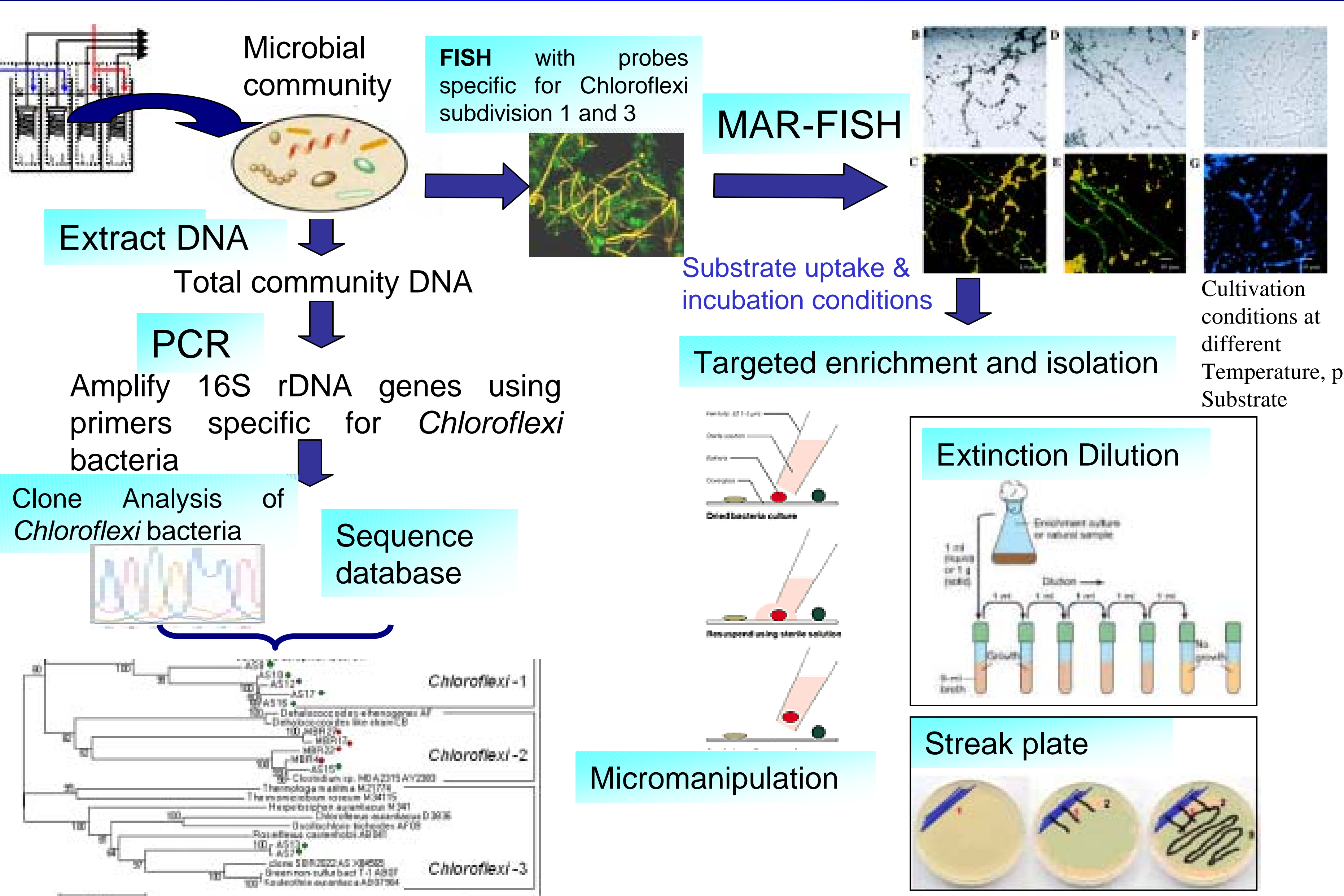
OBJECTIVE 2

Identification and characterization of filamentous *Chloroflexi* in order to study their ecophysiological function in MBRs

PART 2

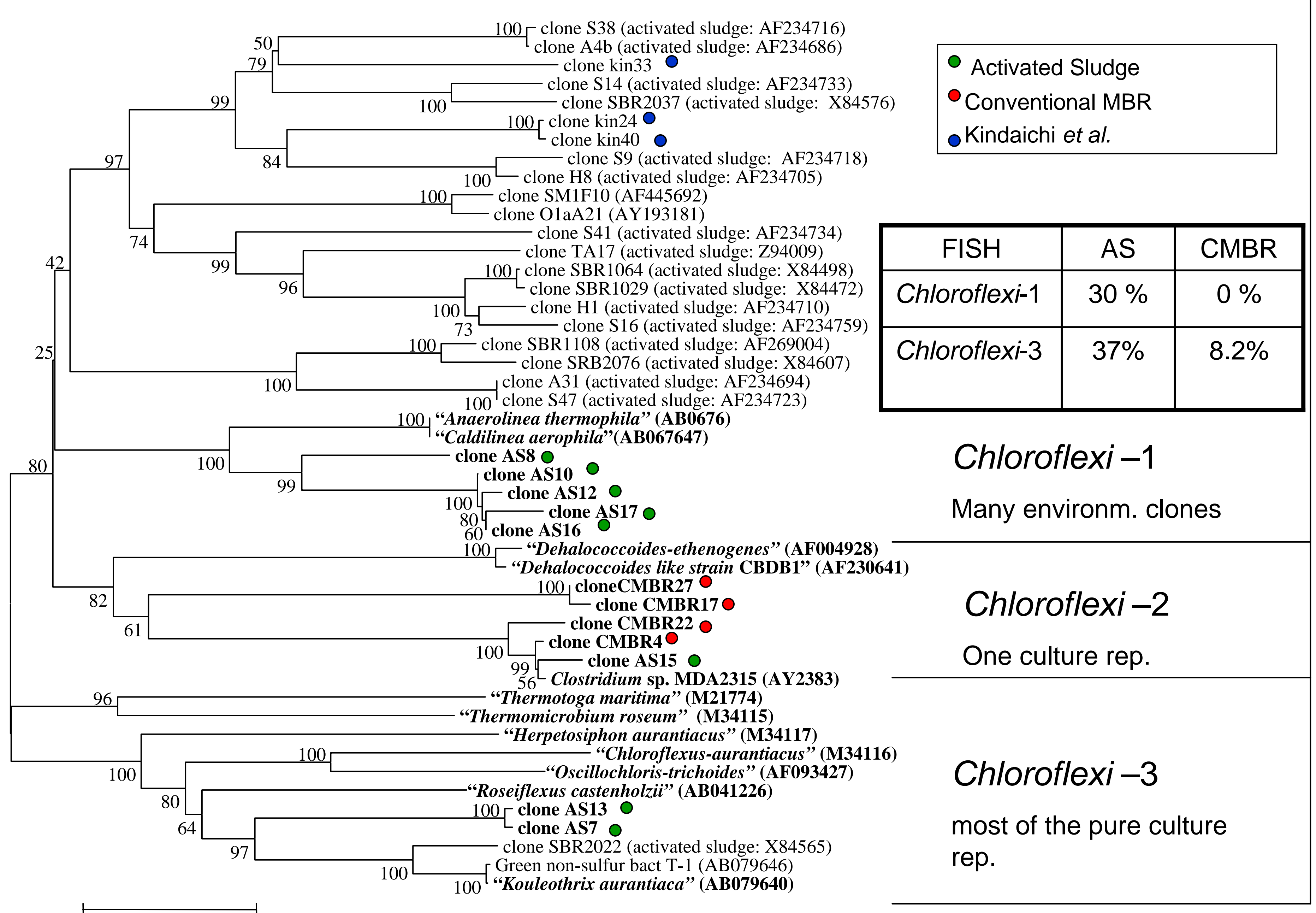
I. Identification and Characterization: 16S rRNA clone analysis & FISH quantitative analysis in subdivision level

II. Characterization: Influence of temperature, oxygen and substrates uptake (MAR-FISH analysis)

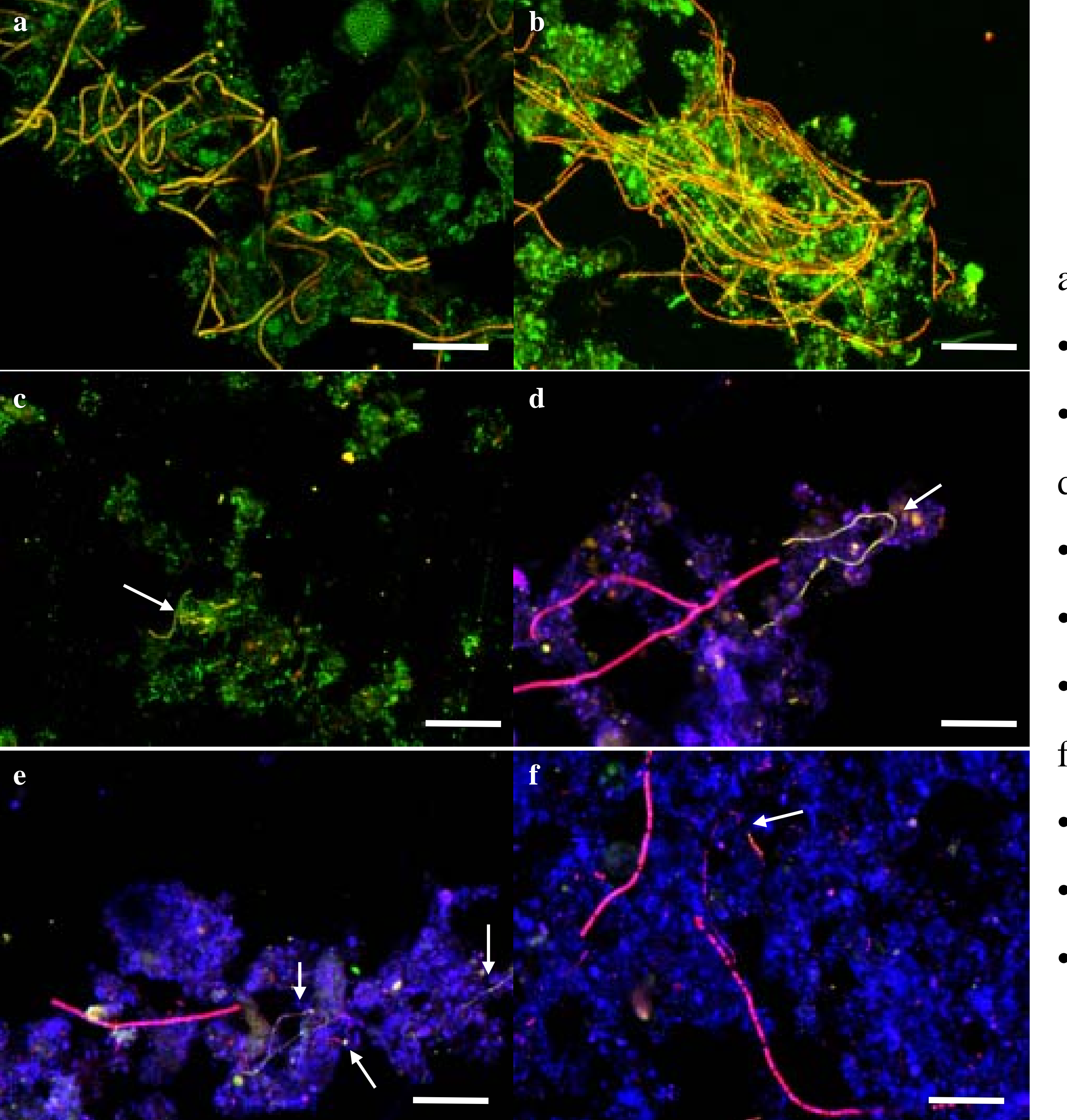


Microbial community
Extract DNA
Total community DNA
PCR
Amplify 16S rDNA genes using primers specific for *Chloroflexi* bacteria
Clone Analysis of *Chloroflexi* bacteria
Sequence database
FISH with probes specific for Chloroflexi subdivision 1 and 3
MAR-FISH
Substrate uptake & incubation conditions
Cultivation conditions at different Temperature, pH, Substrate
Targeted enrichment and isolation
Micromanipulation
Extinction Dilution
Streak plate
Chloroflexi-1
Chloroflexi-2
Chloroflexi-3
Characterization of individual clones
Function of bacteria in the environment

<RESULTS> Cloning & FISH analysis



FISH	AS	CMBR
Chloroflexi-1	30 %	0 %
Chloroflexi-3	37%	8.2%



Chloroflexi in a group and subdivision level

a) AS, b) CMBR,c)HMBR

- *Chloroflexi* (yellow)
- Other bacteria (green)

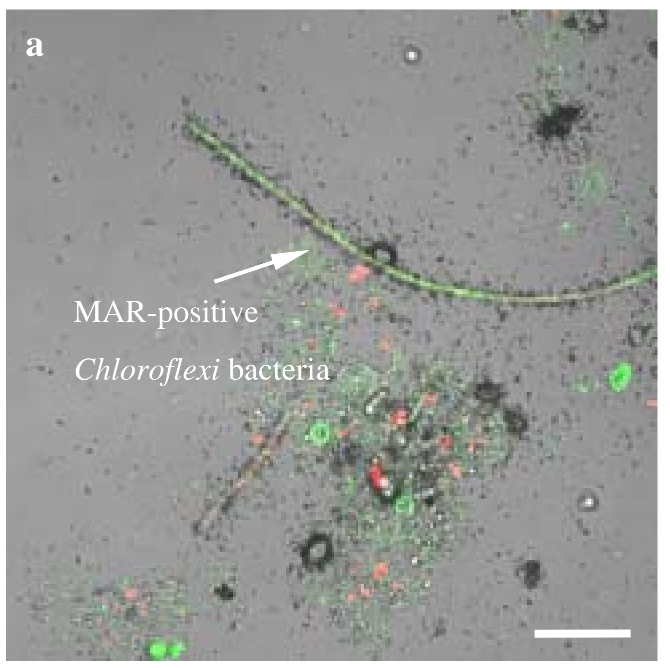
d) AS, e) CMBR

- *Chloroflexi*-1 (yellow)
- *Chloroflexi* (magenta)
- Other bacteria (blue)

f) CMBR

- *Chloroflexi*-3 (yellow)
- *Chloroflexi* (magenta)
- Other bacteria (blue)

<RESULTS> In situ characterization of filamentous *Chloroflexi*



Uptake of N-acetyl-[1-^{14}C] D-Glucosamin, pH 7

Uptake of D-[U-^{14}C] Glucose, pH 10

- *Chloroflexi* bacteria was very active in both oxic and anoxic conditions.
- *Chloroflexi* bacteria is able to uptake N-Acetyl Glucosamin (NAG, major constituent of bacterial cell wall).

< Hypothesis 2 >

The *Chloroflexi* bacteria could grow in the CMBR2 due to:

- They ability to grow in oxic and anoxic conditions.
- They are able to uptake NAG: they are scavengers of dead, dormant, slow growing microorganisms that are presented in more than 40 %.
- The influent (Primary Clarifier effluent) contain large fractions of solid particles and organic matters that are important for the growth of *Chloroflexi* bacteria.

CONCLUSIONS

The importance to study the microbial communities in wastewater treatment is well accepted, however, difficulties still occur in trying to analyze the structure and function of this community and draw conclusions when they are present in effluents from plants treating highly complex feeds. Hence, much of the work regarding microbial communities has been done using defined feeds and pure culture microorganisms.

Today, the molecular methods allow as analyzing in truly cultivation-independent way, the structure of microbial communities.

The molecular methods used in this study revealed that the HMBR microbial community was different from those in the AS and CMBR, and that filamentous *Chloroflexi* seems be a key player in the CMBR performance.

There is still, a long way to understand how the functionally important group of bacteria can be influenced by plant design and how these changes affect process stability.

SOLUTION

Greater collaboration between microbiologist and engineers

Microbiologist generates the information for the process engineers to be able to 'engineer' a solution on the treatment plant scale.

Thank you very much!

CONCLUSIONS

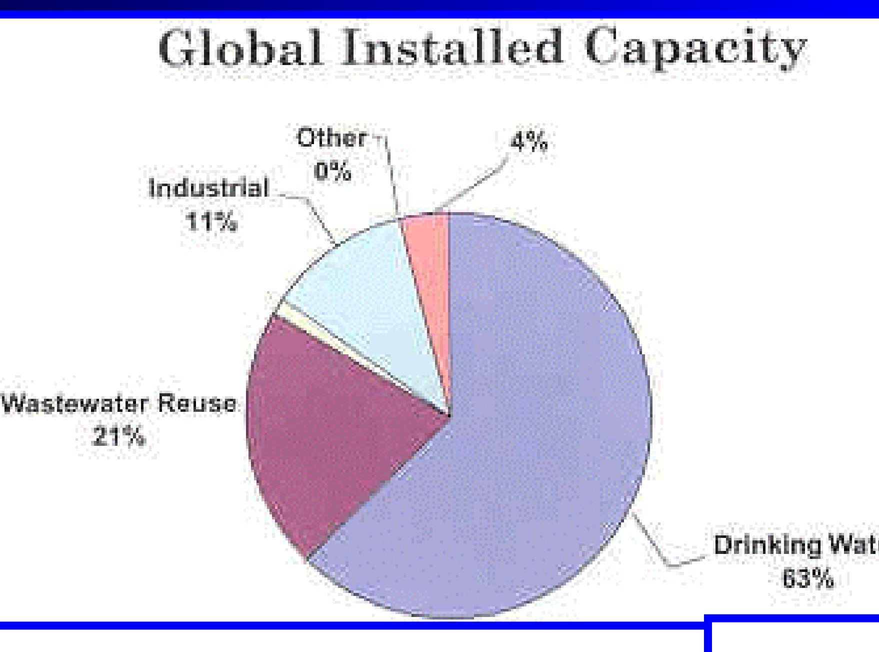
- The microbial community in the hybrid MBRs were different to the conventional MBR and activated sludge process.

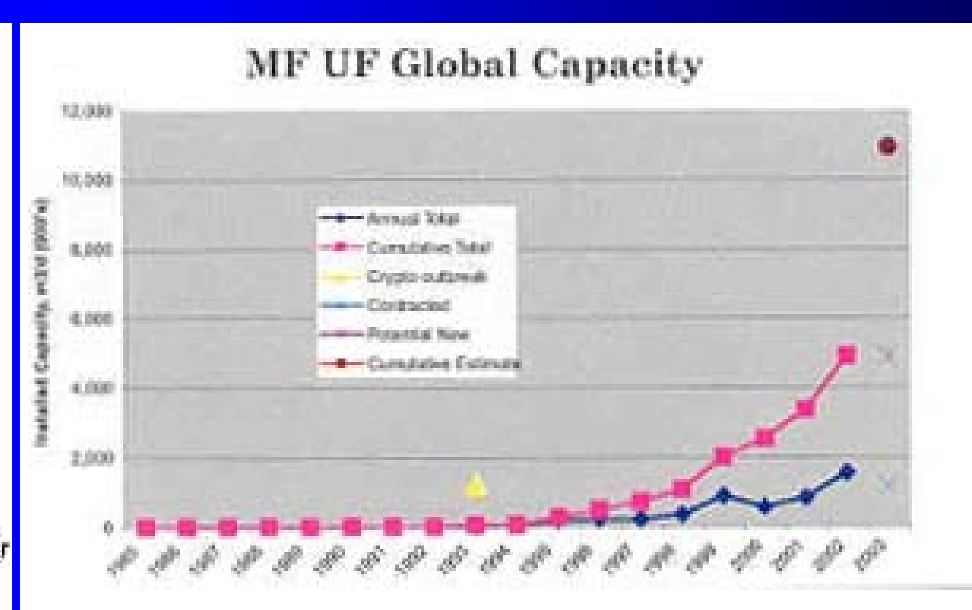
- In the first period operation (rapidly increasing MLSS conc.) the community structure in the hybrid and conventional MBR showed large changes. However, during a stable operation, the hybrid MBR showed large and regular changes in their community structure, while the conventional MBR showed small changes.

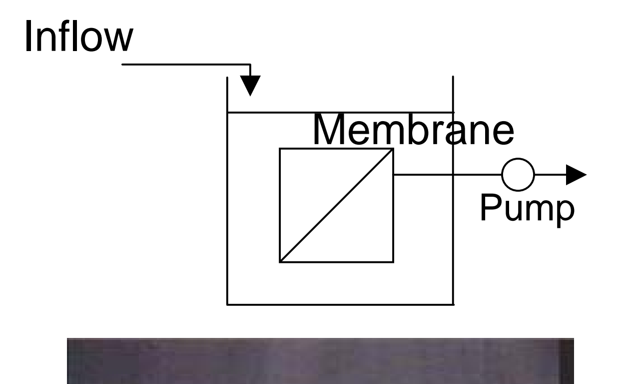
- The four MBRs revealed a very rich community diversity and nearly completely even distribution of species.

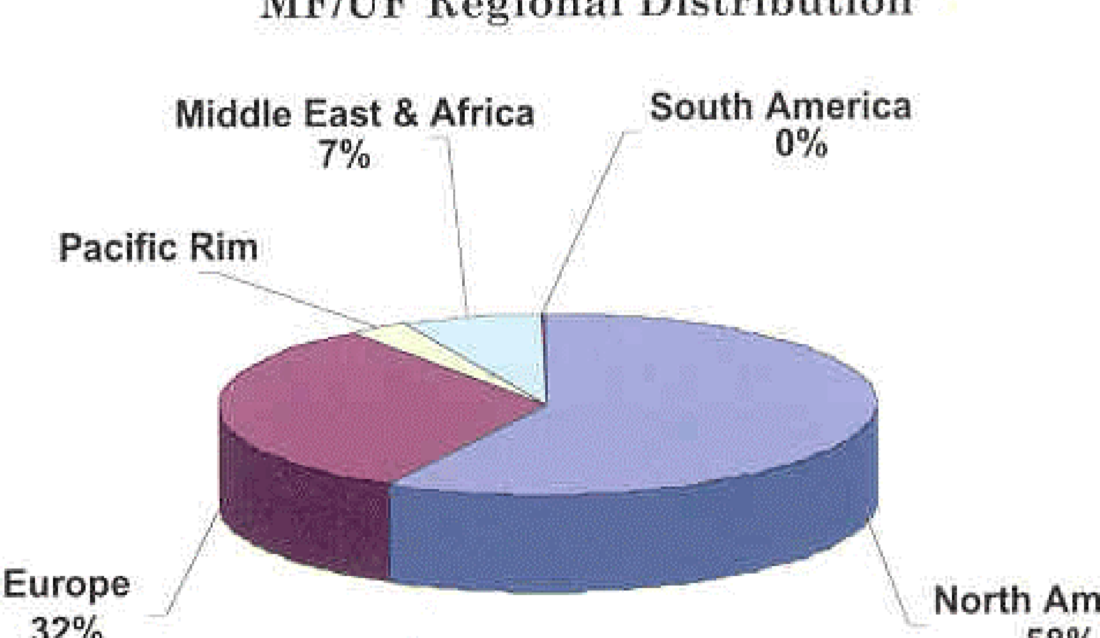
- FISH analysis reveal that the GNS bacteria in the conventional MBR was four times bigger than the hybrid MBR. This filament occurred within the floc and seems to outgrow other bacterial species, producing less dense and large flocs. As a consequence, the mixed liquor viscosity increase, creating conditions for membrane fouling.

GLOBAL STATUS OF MF & UF MEMBRANE

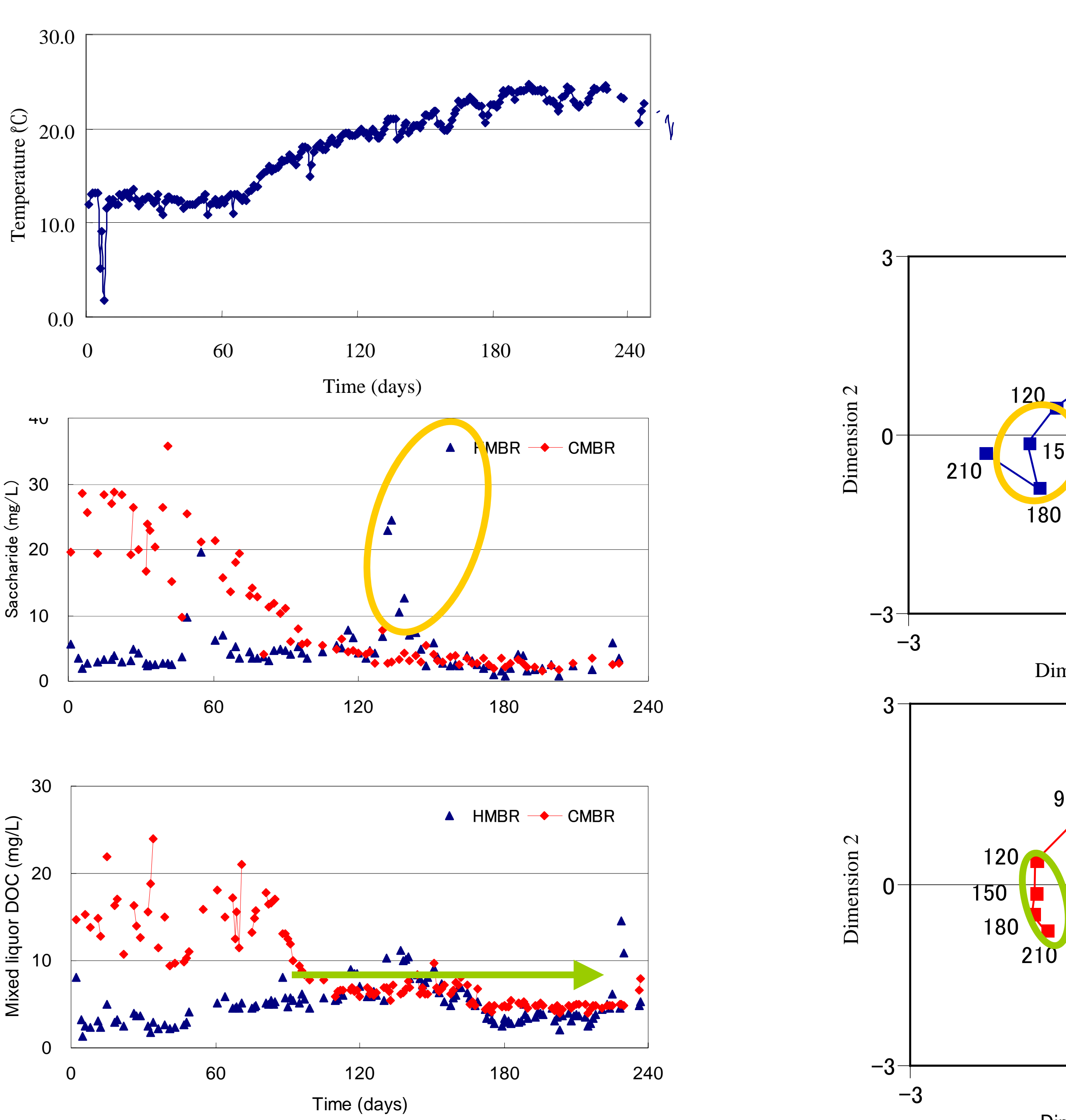


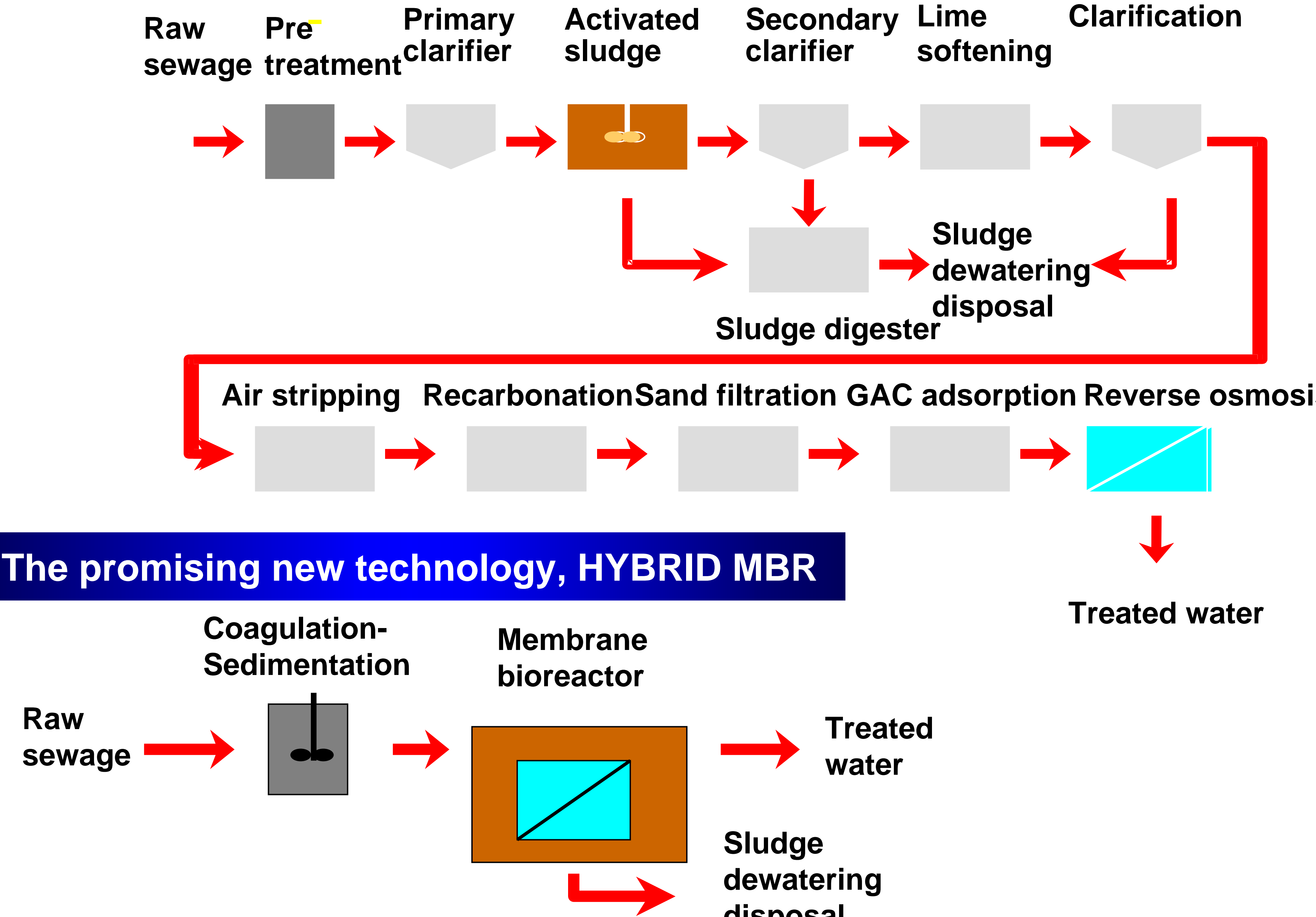




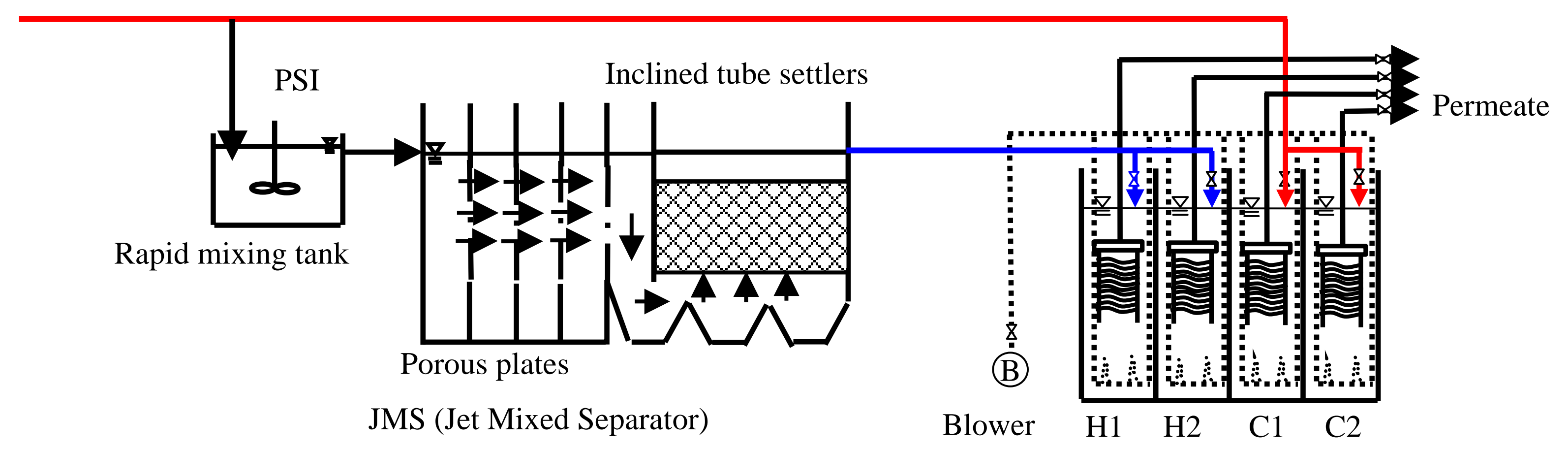


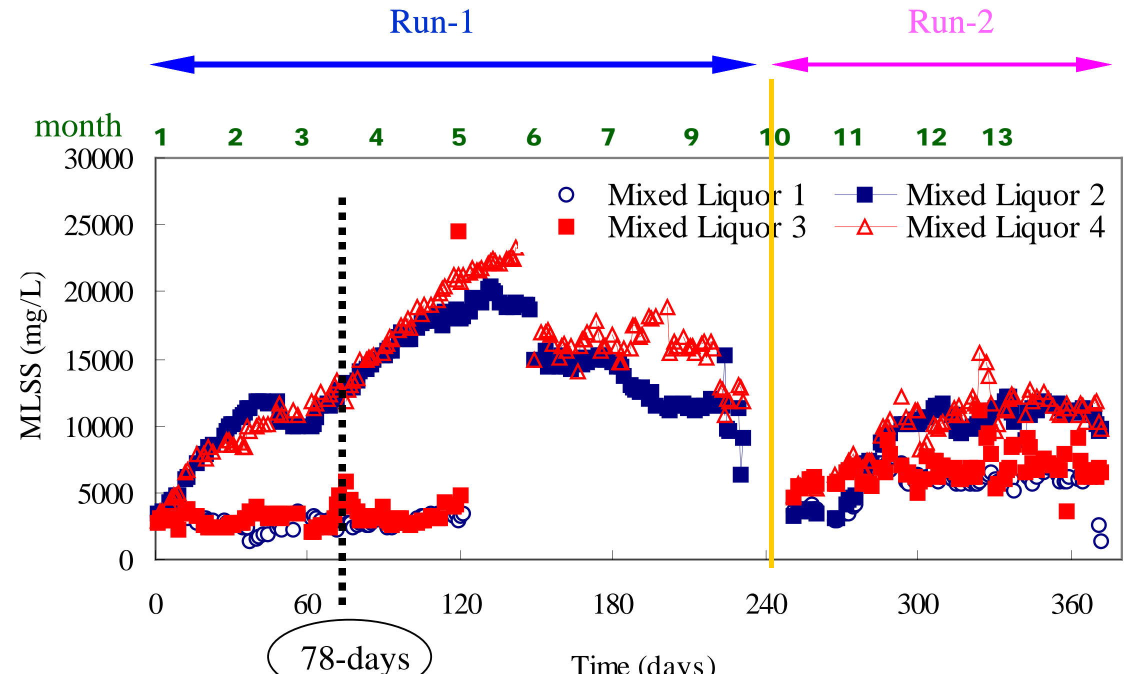
<Results> MBR performance and changes in the microbial community structure




Conventional Advance Wastewater Treatment
Raw sewage
Pre treatment
Primary clarifier
Activated sludge
Secondary clarifier
Lime softening
Clarification
Sludge dewatering disposal
Sludge digester
Air stripping
Recarbonation
Sand filtration
GAC adsorption
Reverse osmosis
Treated water
The promising new technology, HYBRID MBR
Raw sewage
Coagulation-Sedimentation
Membrane bioreactor
Treated water
Sludge dewatering disposal

Process flow schematic of the pilot scale hybrid and conventional MBRs





OPERATION PERIODS

I. Jan, 17 to May, 15 (2002)

MLSS = 2 – 4 (H1 & C1)

MLSS = 2 – 20 (H2 & C2)

II. Jun to Aug (2002)

MLSS = 15 (H2 & C2)

III. Sep, 18 to Jan, 16 2003

MLSS = 5 – 7 (H1 & C1)

MLSS = 10 – 15 (H2 & C2)

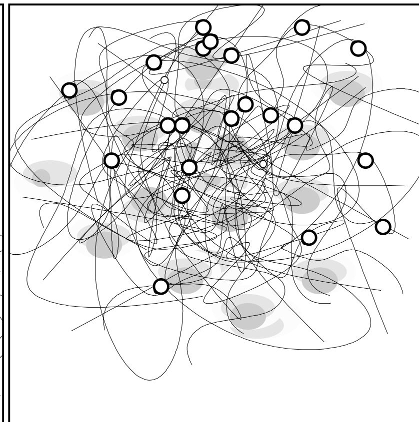
……. Anoxic condition ………. ……….Oxic condition ………..

HMBR CMBR FMBR

HMBR CMBR FMBR

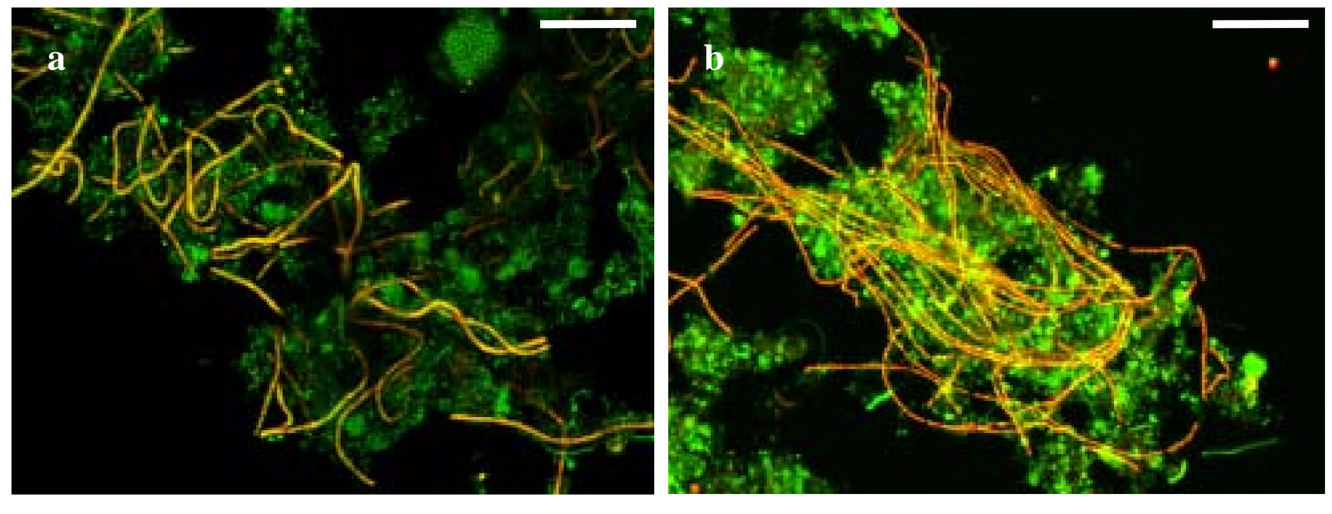
Poor settling characteristics

Filamentous sludge with gas bubbles



Filamentous sludge

Chloroflexi bacteria



Activated sludge CMBR

Actinobacteria

a b c

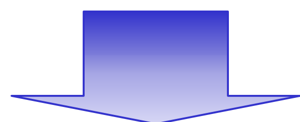
Activated sludge CMBR HMBR

The importance of microbial aspects in biological processes

Microorganisms

- ✓ Carbon and nutrient removal from sewage
- ✖ Formation of foam
- ✖ Bulking
- ✖ Outcompeting microorganisms required for nutrient removal

Knowledge of the ecology of microbial community



Reveal factors influencing the efficiency and stability of biological WWTPs

Develop strategies for improved process performance and for future plant optimization

MBRs operating conditions

		HMBR 1	*HMBR 2*	*CMBR 1*	*CMBR 2*
Influent		JMS effluent		Primary clarifier effluent	
Membrane pore size	RUN1	0.4	0.4	0.4	0.4
(μm)	RUN2	0.2	0.2	0.2	0.2
HRT	RUN1	4.5-6.0	3.6-4.5	4.5-6.0	3.6-6.0
(h)	RUN2	3.6-4.5	3.6-4.5	3.6-4.5	3.6-4.5
Flux	RUN1	0.3-0.4	0.4-0.5	0.3-0.4	0.3-0.5
($m.day^{-1}$)	RUN2	0.4-0.5	0.4-0.5	0.4-0.5	0.4-0.5
MLSS	RUN1	2-3	15-25	2-3	15-25
($g.L^{-1}$)	RUN2	5-6	10-12	5-6	10-12

Physicochemical characteristics of the Primary Clarifier (PC) and JMS effluent

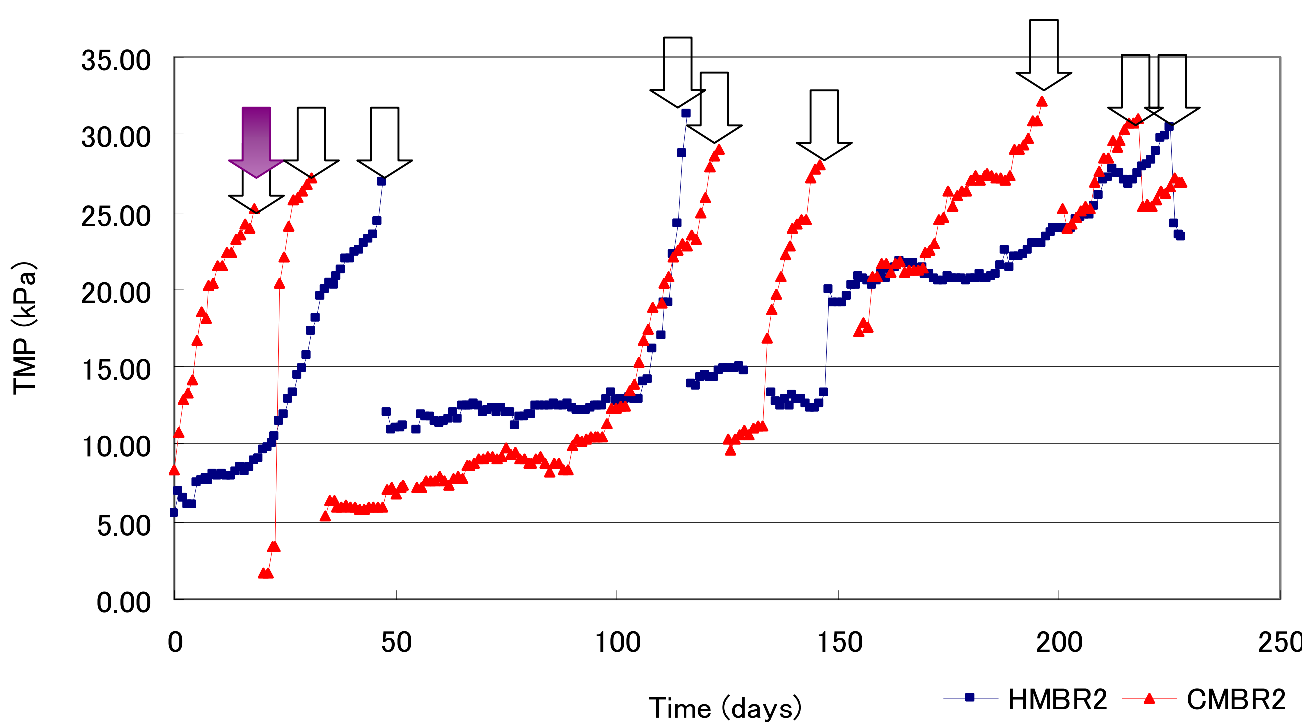
		RUN1		*RUN2*	
Influent		PC effluent	JMS effluent	PC effluent	JMS effluent
Temperature	℃	17.8	17.8	13.1	13.1
pH	$mg.L^{-1}$	7.7	6.8	7.6	7.0
DO	NTU	1.7	1.9	1.9	1.9
Turbidity	$mg.L^{-1}$	53.2	9.0	50.2	12.5
TOC	$mg.L^{-1}$	35.3	16.7	35.8	22.7
DOC	$mg.L^{-1}$	20.6	12.9	21.3	17.6
T-N	$mg.L^{-1}$	29.7	20.7	26.3	24.1
T-P	$mg.L^{-1}$	2.6	0.5	2.3	0.8

MBR performance

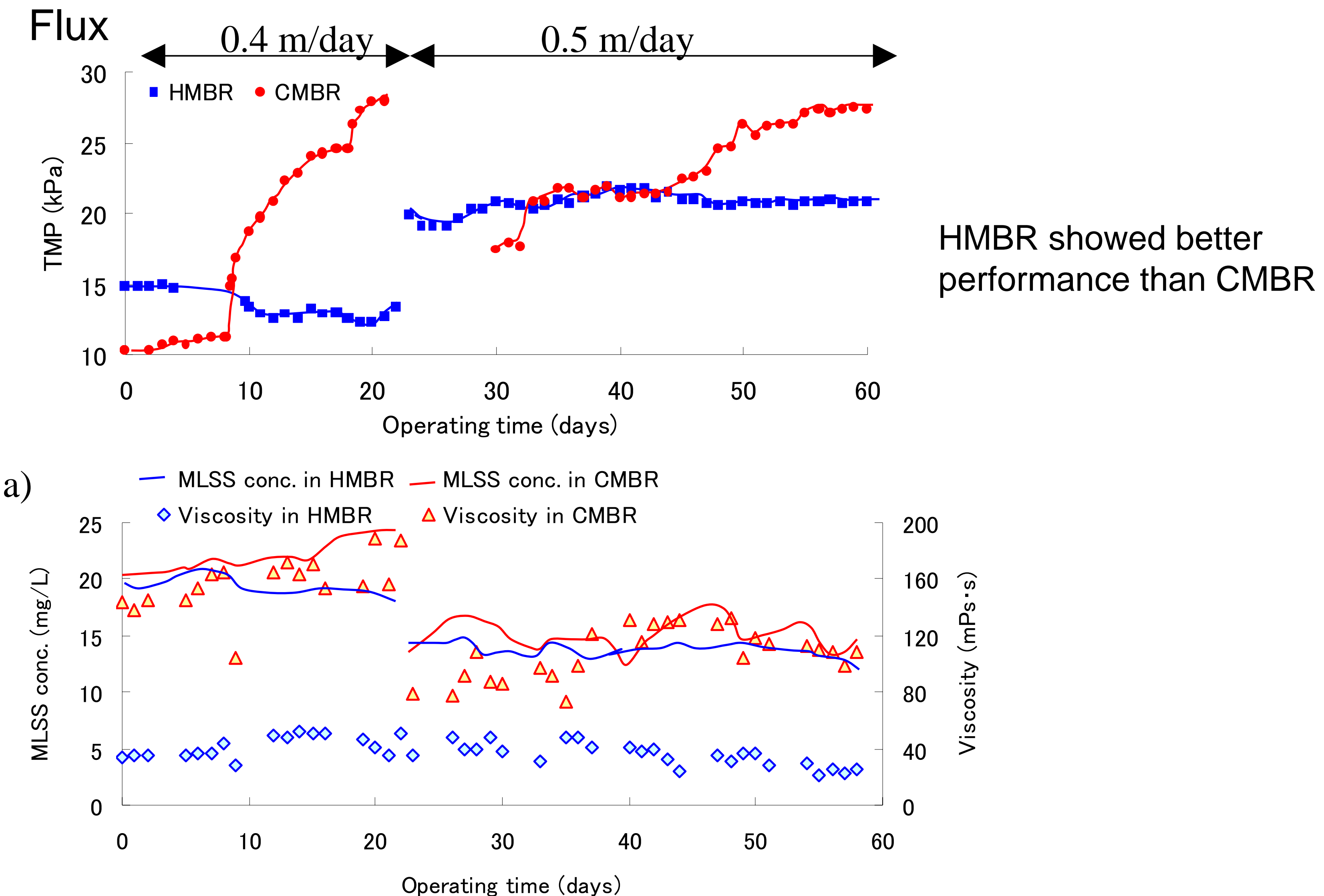
Permeate water quality

Parameters	*Unit*	*PC effluent*	*JMS effluent*	*HMBR 1*	*HMBR 2*	*CMBR 1*	*CMBR 2*
Turbidity	NTU	53.8	8.0	0	0	0	0
DOC	$mg.L^{-1}$	19.1	12.9	4.6	3.2	5.0	4.2
E260	$1.cm^{-1}$	0.21	0.19	0.08	0.07	0.11	0.10
Total phosphorus	$mg.L^{-1}$	2.06	0.36	0.03	0.03	0.44	0.68
Total nitrogen	$mg.L^{-1}$	25.4	17.9	16.9	16.5	18.8	18.4
NH_4^+-N	$mg.L^{-1}$	13.6	12.1	3.1	1.8	0.7	0.6
NO_2^--N	$mg.L^{-1}$	0.1	0.0	1.5	0.1	0.9	0.1
NO_3^--N	$mg.L^{-1}$	0.7	0.5	11.2	14.1	14.7	14.9
Alkalinity	$mg.L^{-1}$	127.6	87.5	8.8	4.0	20.7	27.0
pH		7.1-8.5	6.3-7.2	4.3-7.4	4.0-7.0	6.3-7.4	6.0-7.6

Membrane permeability



MBR performance (membrane permeability) and Sludge characteristics < Itonaga *et al.* >

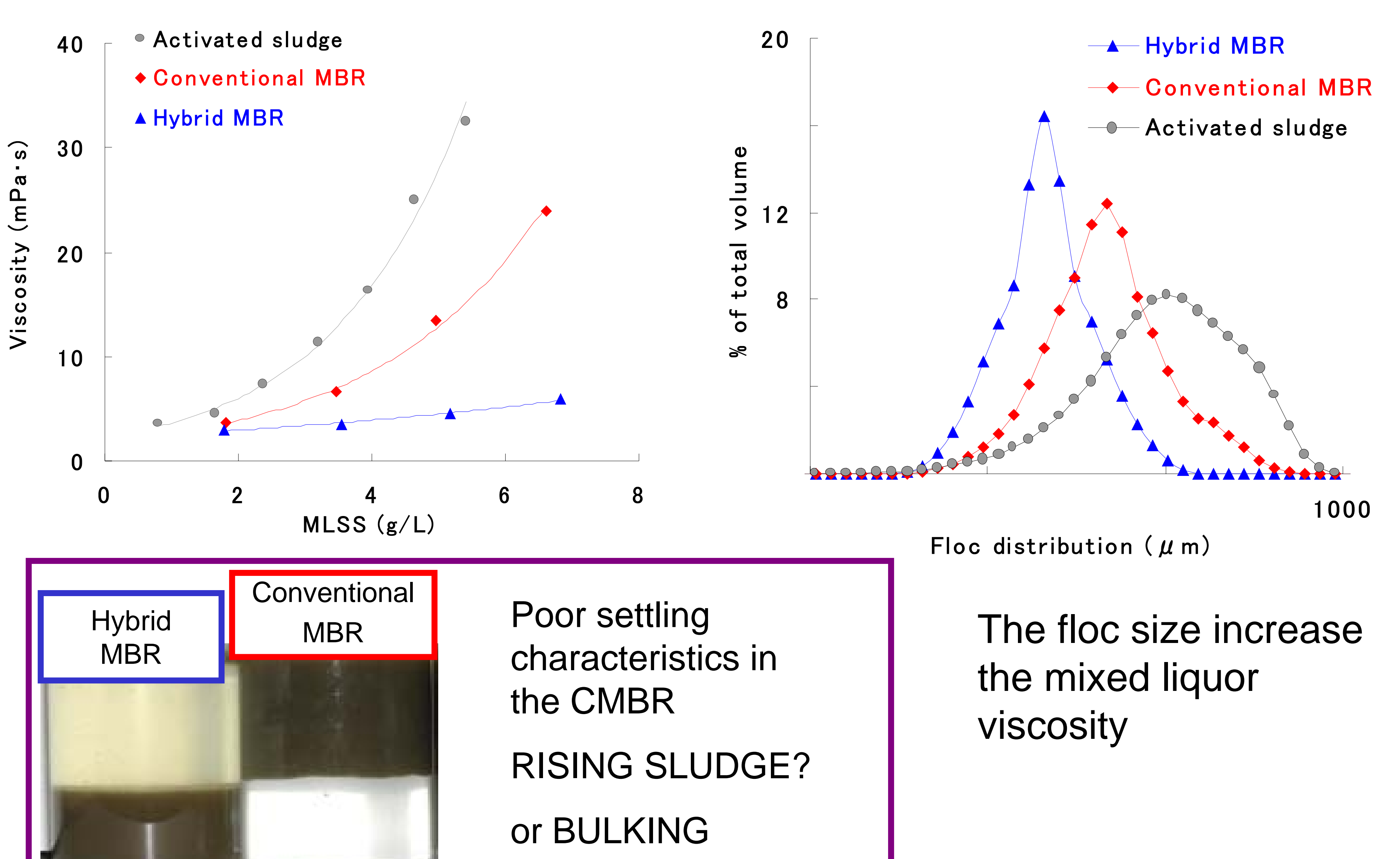


HMBR showed better performance than CMBR

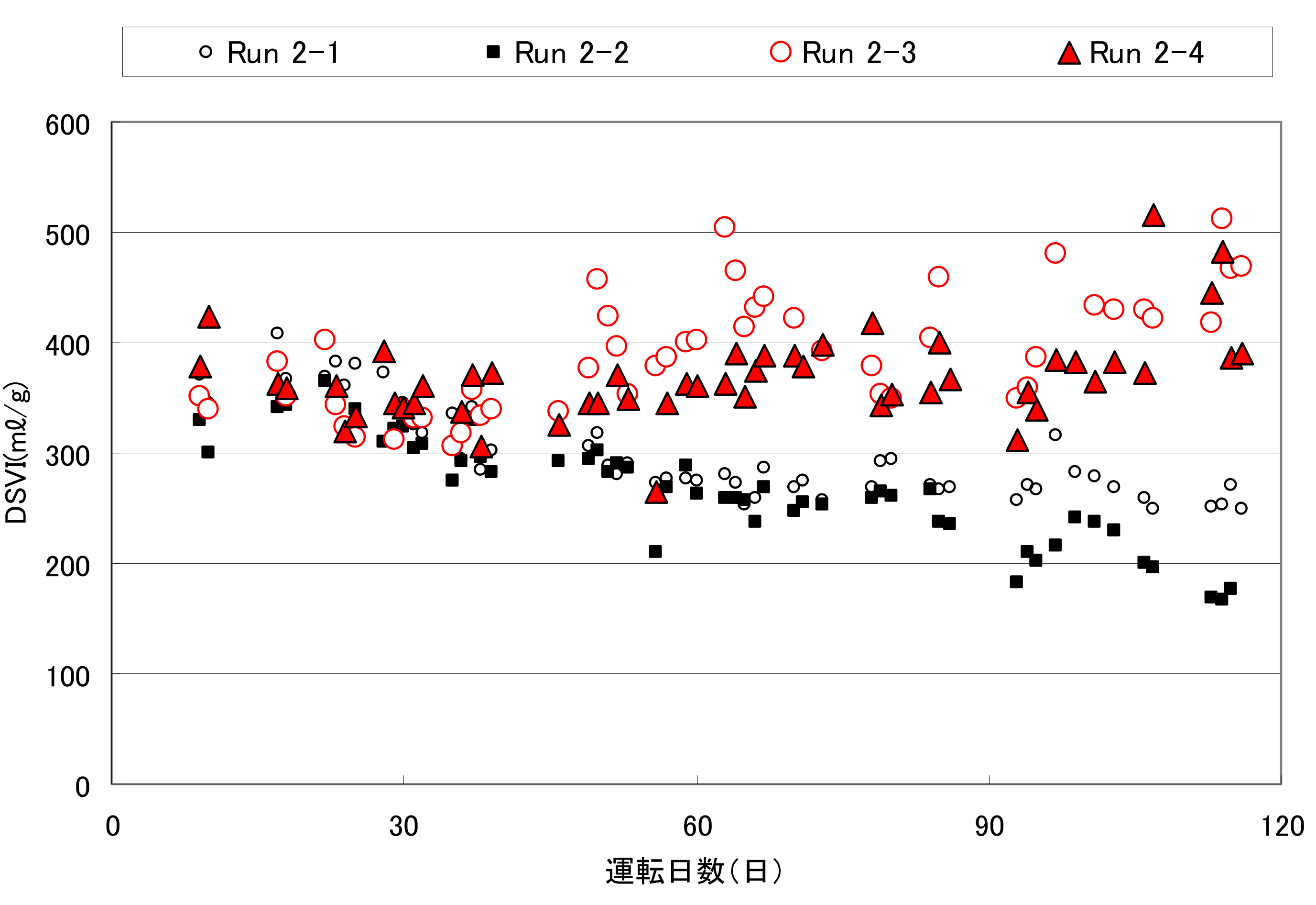
The viscosity in CMBR was very high comparing with the HMBR operating at the same MLSS concentration

Relationship between MLSS conc., viscosity and floc distribution

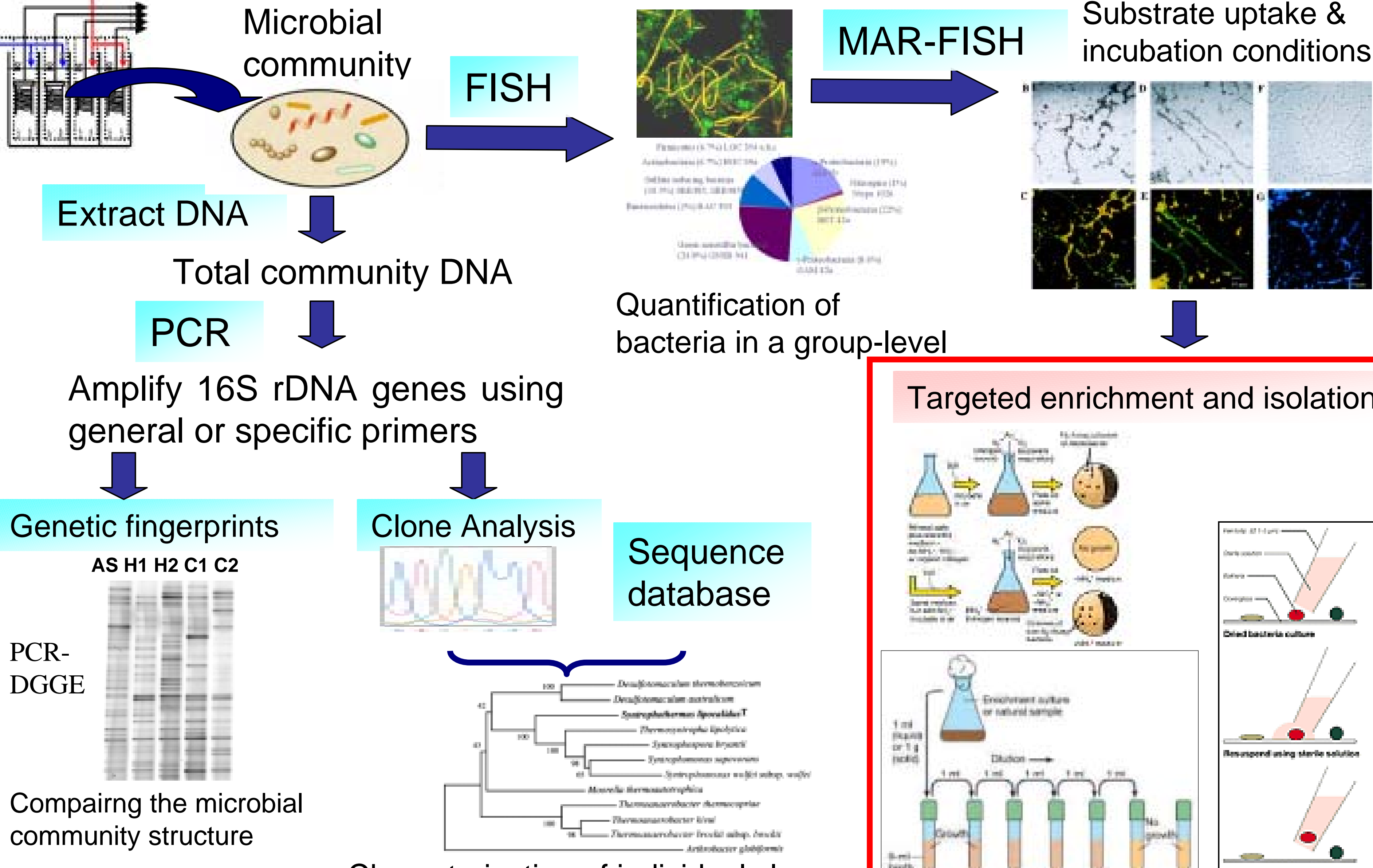
<Itonaga *et al.*>



Sludge Volume Index

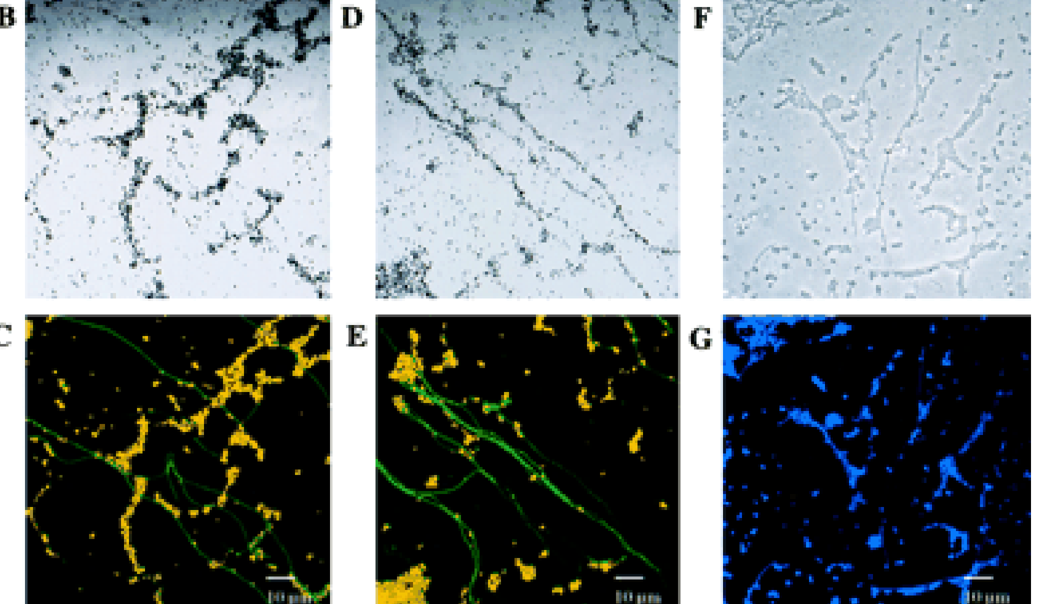


Flow diagram showing the different steps to study the microbial community

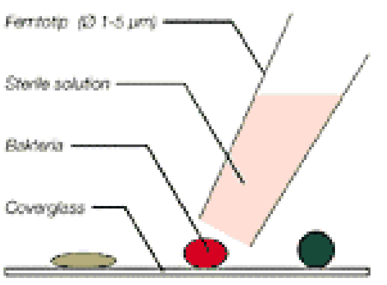


<FUTURE PROSPECT> Chloroflexi bacteria enrichment and isolation

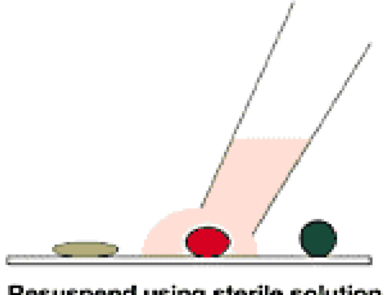
MAR-FISH analysis

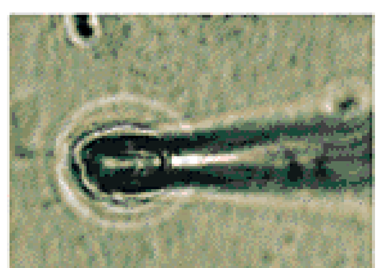


Micromanipulation

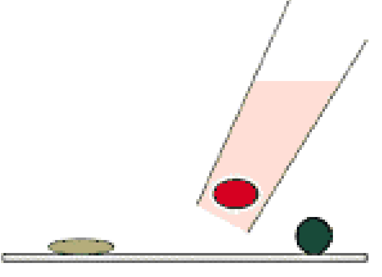


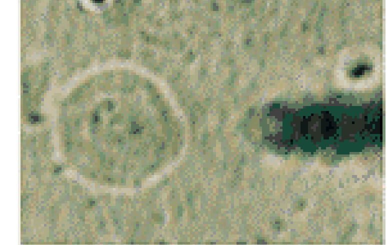
Dried bacteria culture





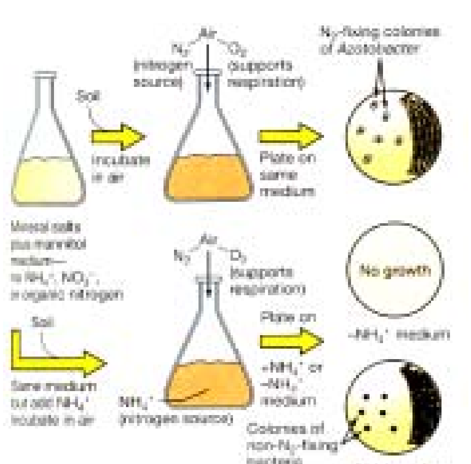
Resuspend using sterile solution



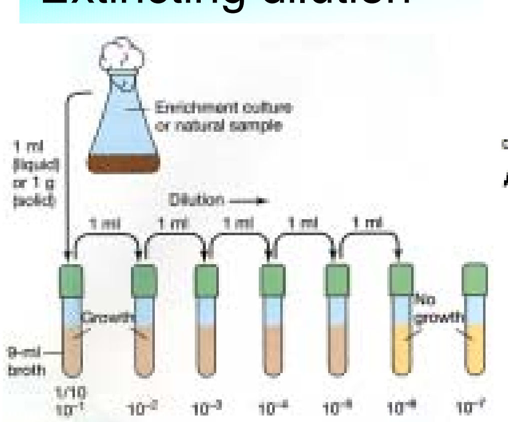


Aspirate cell suspension

Enrichment culture & streak plate

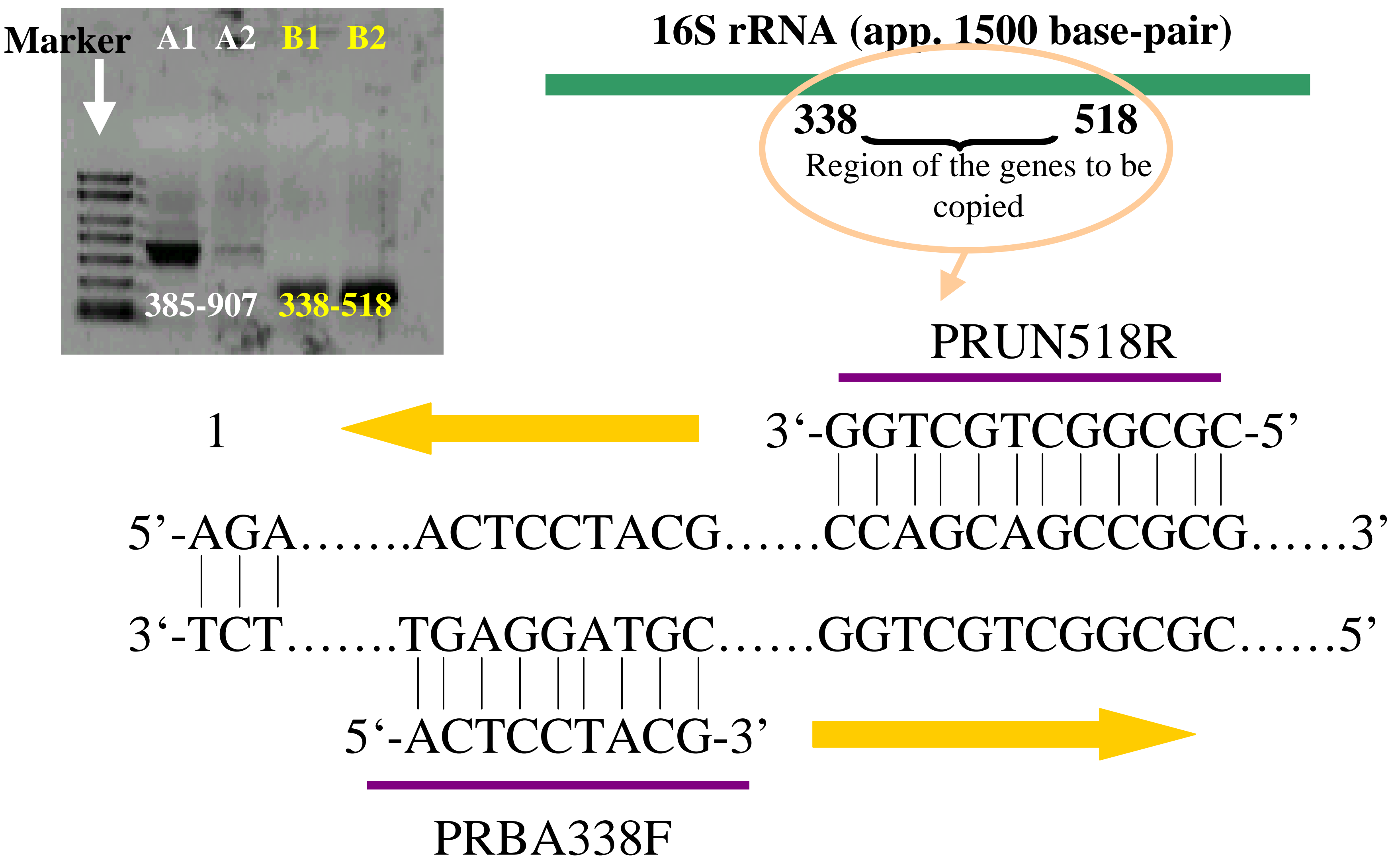


Extincting dilution

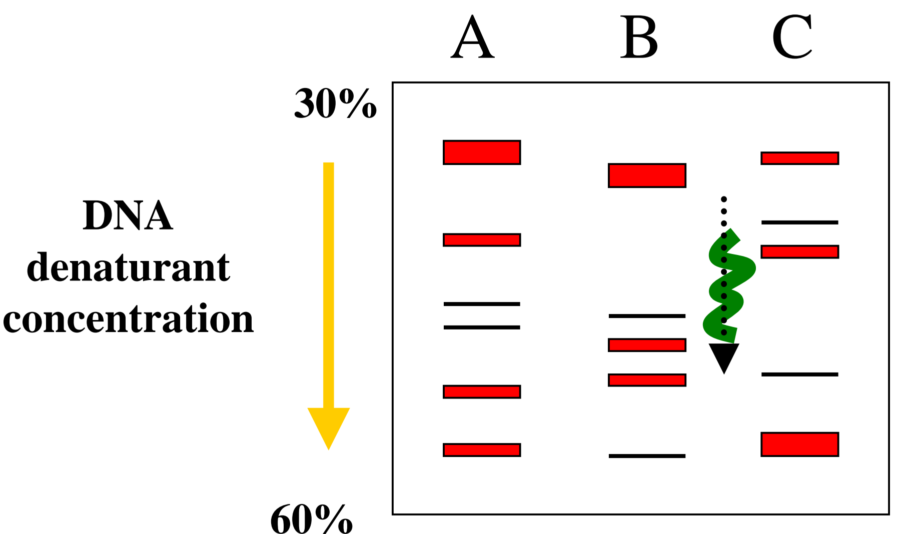


Polymerase Chain Reaction (PCR)

amplification of genes encoding the 16S rRNA



Denaturing Gradient Gel Electrophoresis (DGGE)



Separation of DNA fragments in DGGE is based on the electrophoretic mobility of partially melted double-stranded DNA molecules in polyacrylamide gels containing a linear gradient of denaturants . Molecules with different sequences may have a different melting behavior, and will, therefore, stop migrating at different positions in the gel.

Dice coefficient (Cs)

$$Cs = \frac{2j}{(a+b)}$$

A B

j = 6

Cs = 0.80

a = 8 b = 7

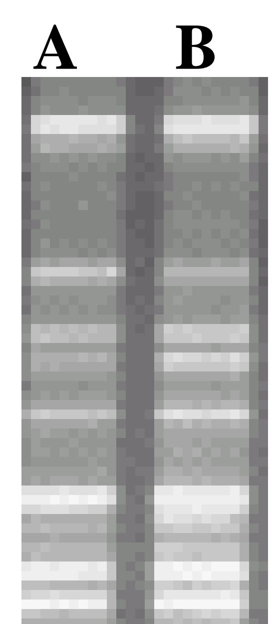
j = number of bands common to samples A and B

a = number of bands in sample A

b = number of bands in sample B

Cs = 0; no common bands

Cs = 1; identical bands patterns



Cs = 1

Shannon diversity index (H) & Equitability index (EI)

$$H = -\sum (ni / N)(\log ni / N)$$

$$EI = H / \log n$$

Each band = single species

Band intensity = species abundance.

***ni* /N = proportion of community that is made up by species *i* (brightness of bands i / total brightness of all bands in the lane)**

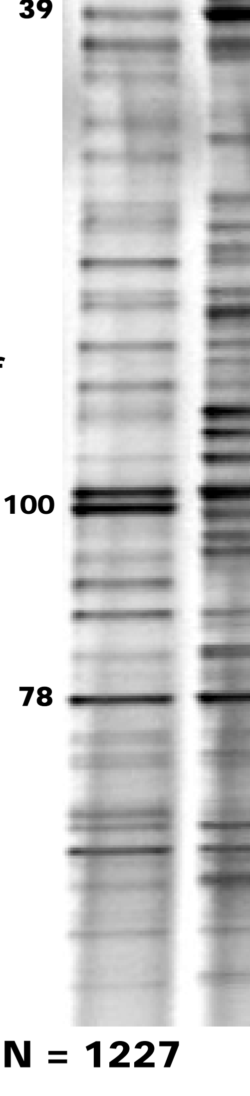
n = total number of species in the sample = total number of bands.

H = Population with more species and even distribution of individuals have higher diversity

EI = evenness of species distribution

EI = 1.0, completely even species distribution

(e.g. 10 species, each at 10% abundance)



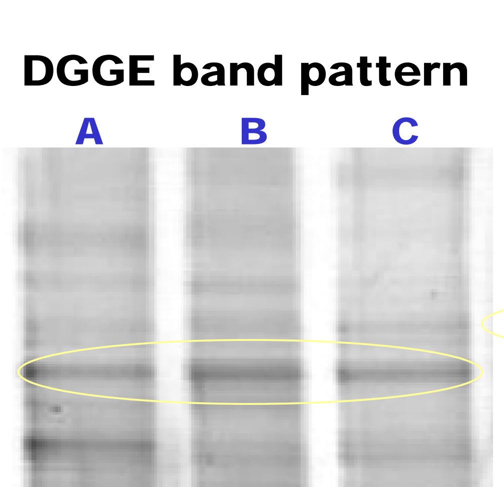
N = 1227

H = 1.41

EI = 0.98

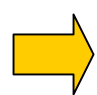
Nonmetric multidimensional scaling (NMDS)

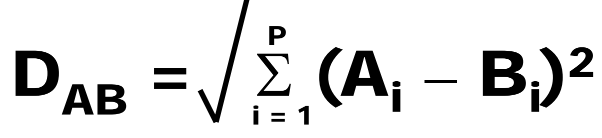
DGGE band pattern



Binary matrix

Band position and intensity		
A	B	C
		38
53		
		73
49		
55	39	100
53	50	71
		56
	37	
35		
26		
35	45	50
		64
84	39	





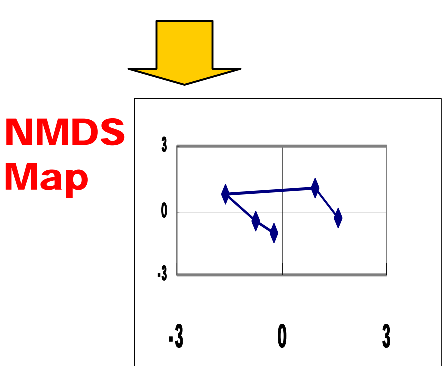
$$D_{AB} = \sqrt{\sum_{i=1}^{P} (A_i - B_i)^2}$$

Distance matrix

	17-Jan	15-Feb	20-Mar	18-Apr	15-May
17-Jan					
15-Feb	267.2				
20-Mar	331.1	308.2			
18-Apr	378	332.6	297.5		
15-May	380.2	379	308.1	326.5	
18-Jun	395.5	394.5	328.7	351.4	216

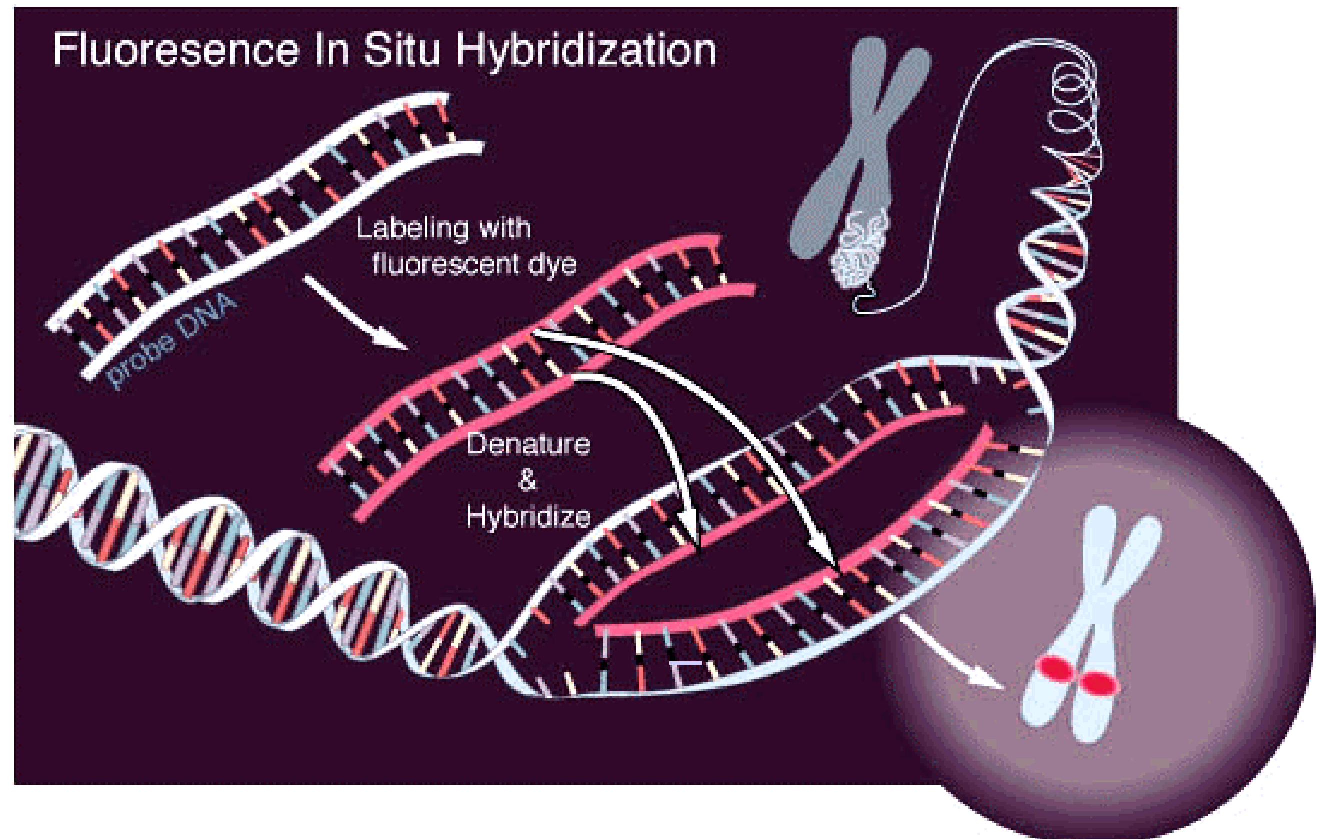
Analyze by NMDS using SPSS 11.5 for Windows

NMDS Map



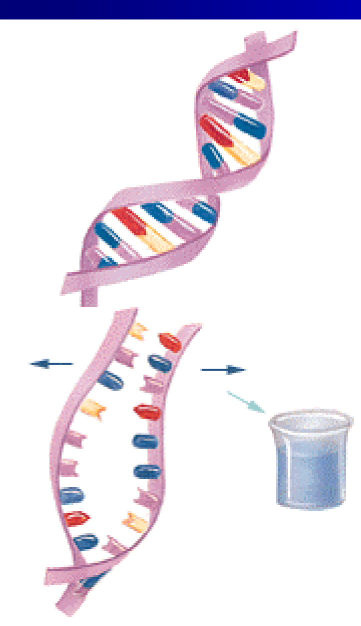
The NMDS map shows the community structure at a particular point in time as one dated point, and by connecting consecutive points, relative changes in the community can be visualized and interpreted.

Fluorescence *in situ* hybridizations (FISH)



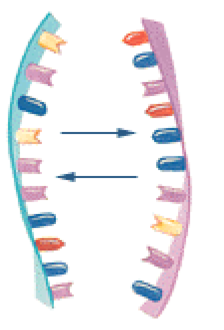
Fluorescent In Situ Hybridization (FISH) is a technique used for the detection of target DNR or RNA molecules with a system of coupled fluorochromes. The detection of nucleotidic sequences on a combed DNA molecule is performed indirectly, by first hybridizing the seeked nucleotidic sequences with the combed DNA (also called the matrix DNA or target).

DNA-chips technology



Step 1: Determine chemical structure of fragment.
Representing all or part of a DNA strand of interest, short fragments of DNA (typically involving 5–25 base pairs) are identified.

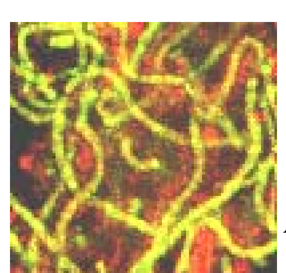
Step 2: Separate strands.
DNA is denatured (separated) and placed in solution or on a solid substrate, forming a reference segment for the DNA fragment of interest.

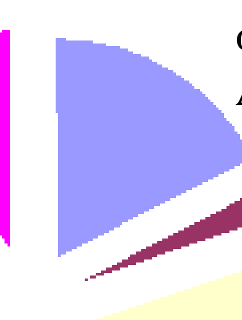


Step 3: Introduce sample.
Unknown DNA sample is introduced to the reference segment. If present, the complement of the reference segment will hybridize (bond) to it.

Step 4: Identify result.
Chemicals that bond to successful hybridization help researchers identify results. Such chemicals are typically photosensitive (fluorescent or chemiluminescent), which helps researchers confirm results.

Activated Sludge



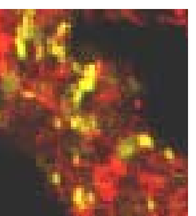


Other bacteria (10%)

Firmicutes (2.7%)
LGC354a,b,c

Actinobacteria (14.1%)
HGC69a

Sulfate reducing bacteria (5.5%)
SRB385, SRB385Db



Bacteroidales (1.5%
BAC303

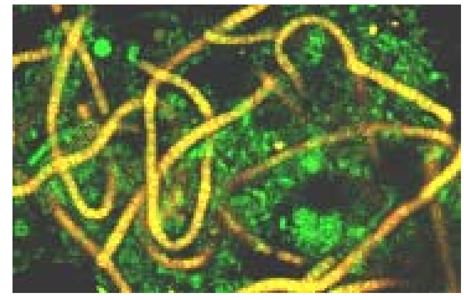
Chloroflexi (11.6%)
GNSB941, CFX1223

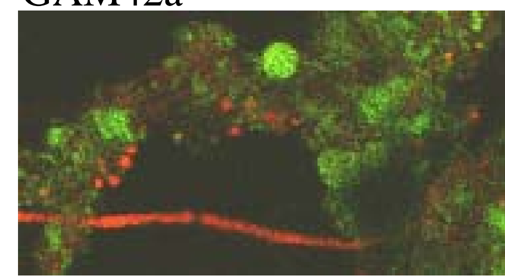
α-*Proteobacteria* (17.7%)
ALF1b

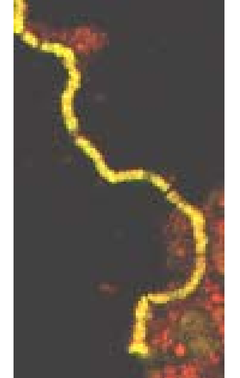
Nitrospira (2.3%)
Ntspa1026

β-*Proteobacteria* (24.7%)
BET42a

γ-*Proteobacteria* (9.9%)
GAM42a







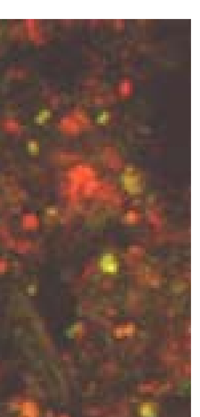
Conventional MBR

Other bacteria (9%)

Firmicutes (4.7%)
LGC354a,b,c

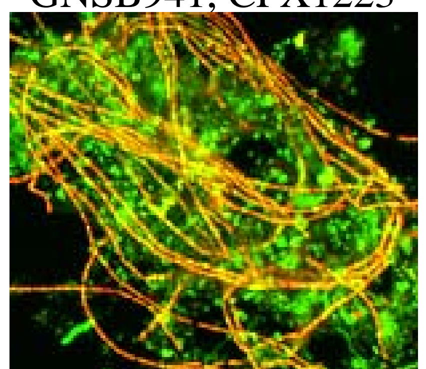
Actinobacteria (6.9%)
HGC69a

Sulfate reducing bacteria (10%)
SRB385, SRB385Db



Bacteroidales (0.7%)
BAC303

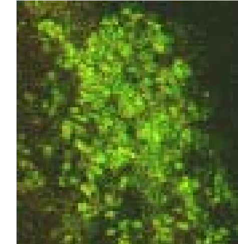
Chloroflexi (19.2%)
GNSB941, CFX1223



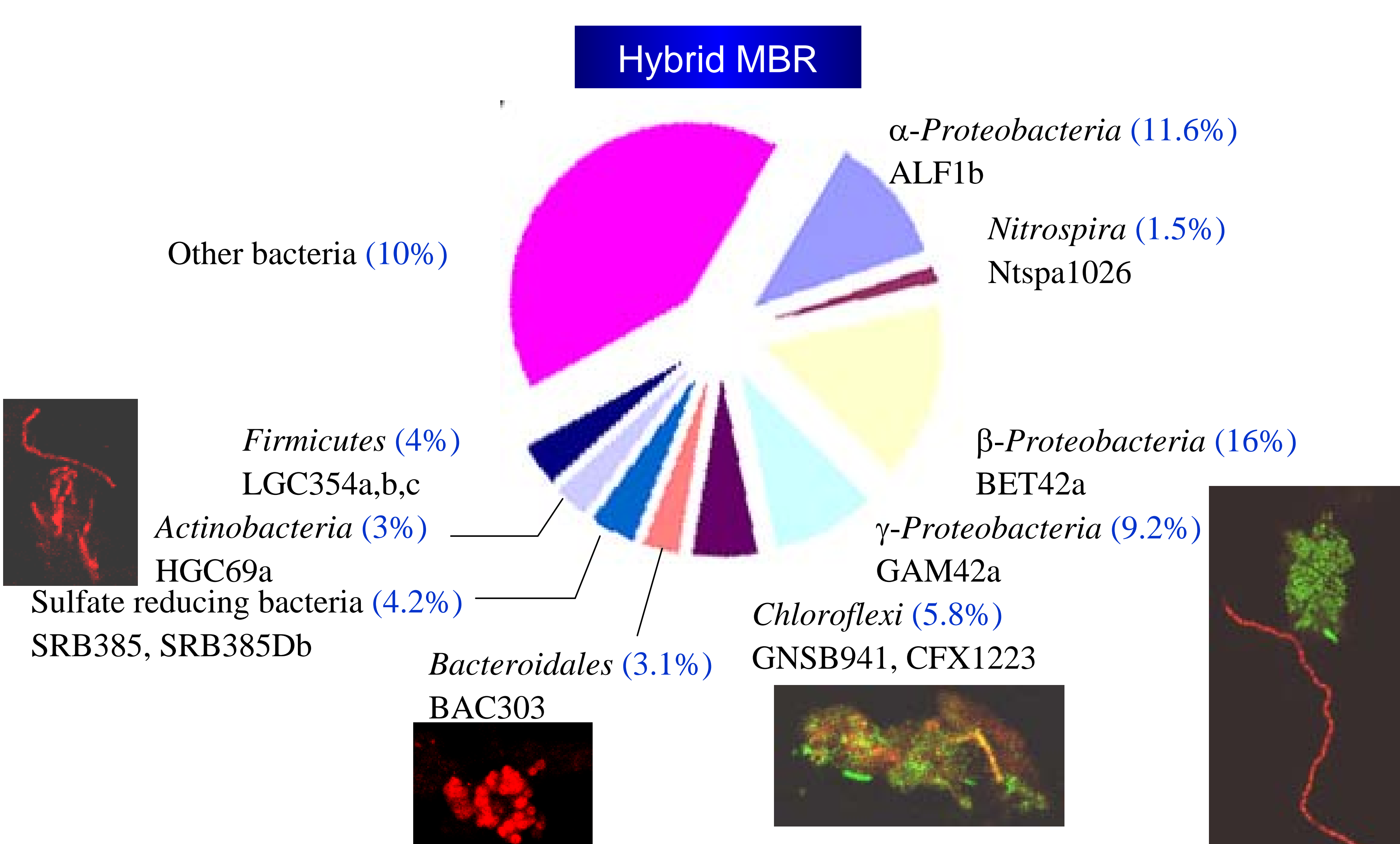
α-*Proteobacteria* (16.3%)
ALF1b

Nitrospira (1.2%)
Ntspa1026

β-*Proteobacteria* (23%)
BET42a

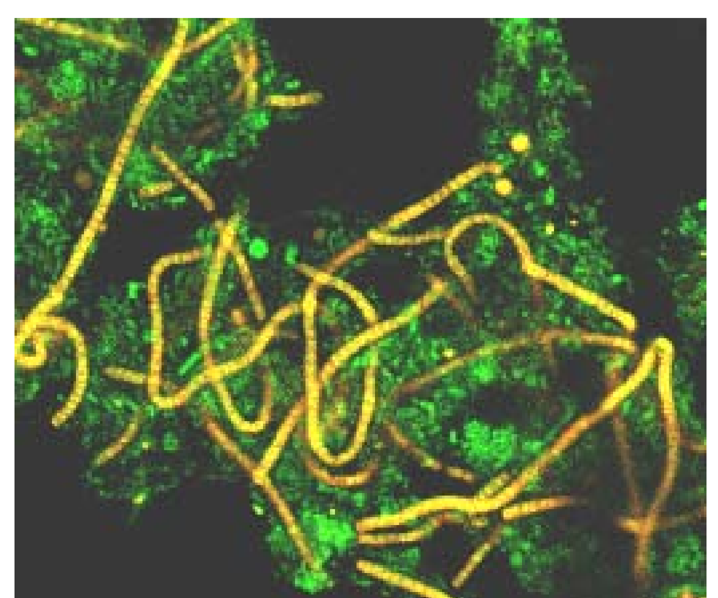


γ-*Proteobacteria* (9%)
GAM42a

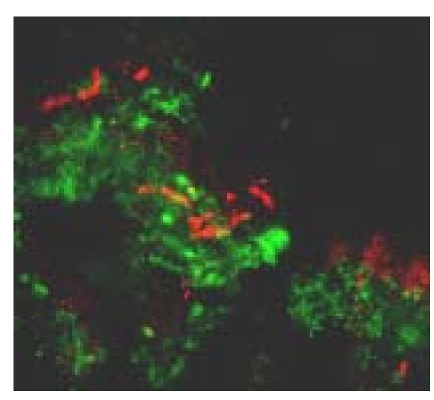


Hybrid MBR
α-Proteobacteria (11.6%)
ALF1b
Nitrospira (1.5%)
Ntspa1026
Other bacteria (10%)
β-Proteobacteria (16%)
BET42a
Firmicutes (4%)
LGC354a,b,c
Actinobacteria (3%)
HGC69a
γ-Proteobacteria (9.2%)
GAM42a
Sulfate reducing bacteria (4.2%)
SRB385, SRB385Db
Chloroflexi (5.8%)
GNSB941, CFX1223
Bacteroidales (3.1%)
BAC303

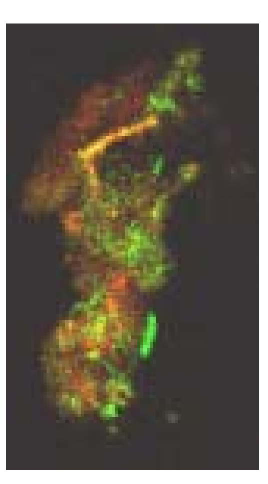
Chloroflexi bacteria



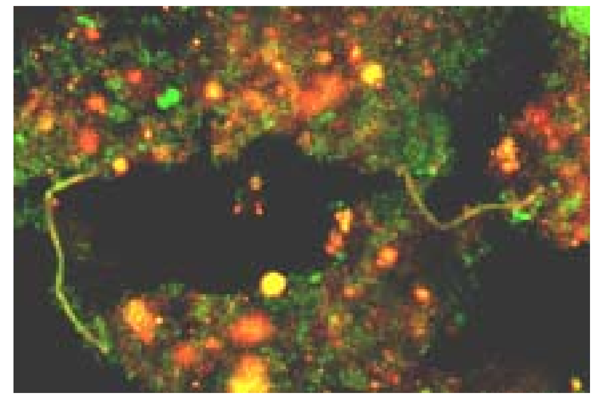
Activated Sludge (11%)



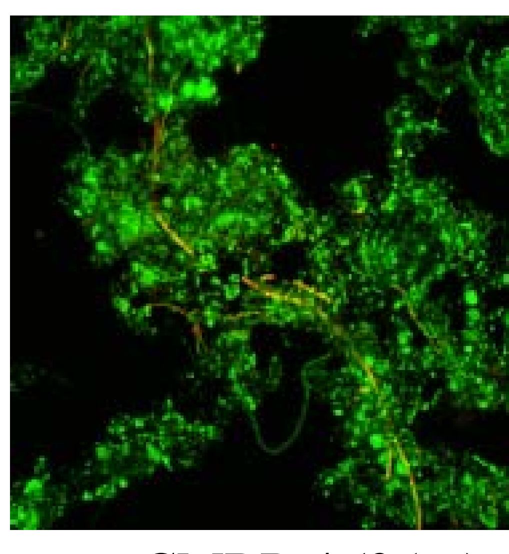
HMBR 1



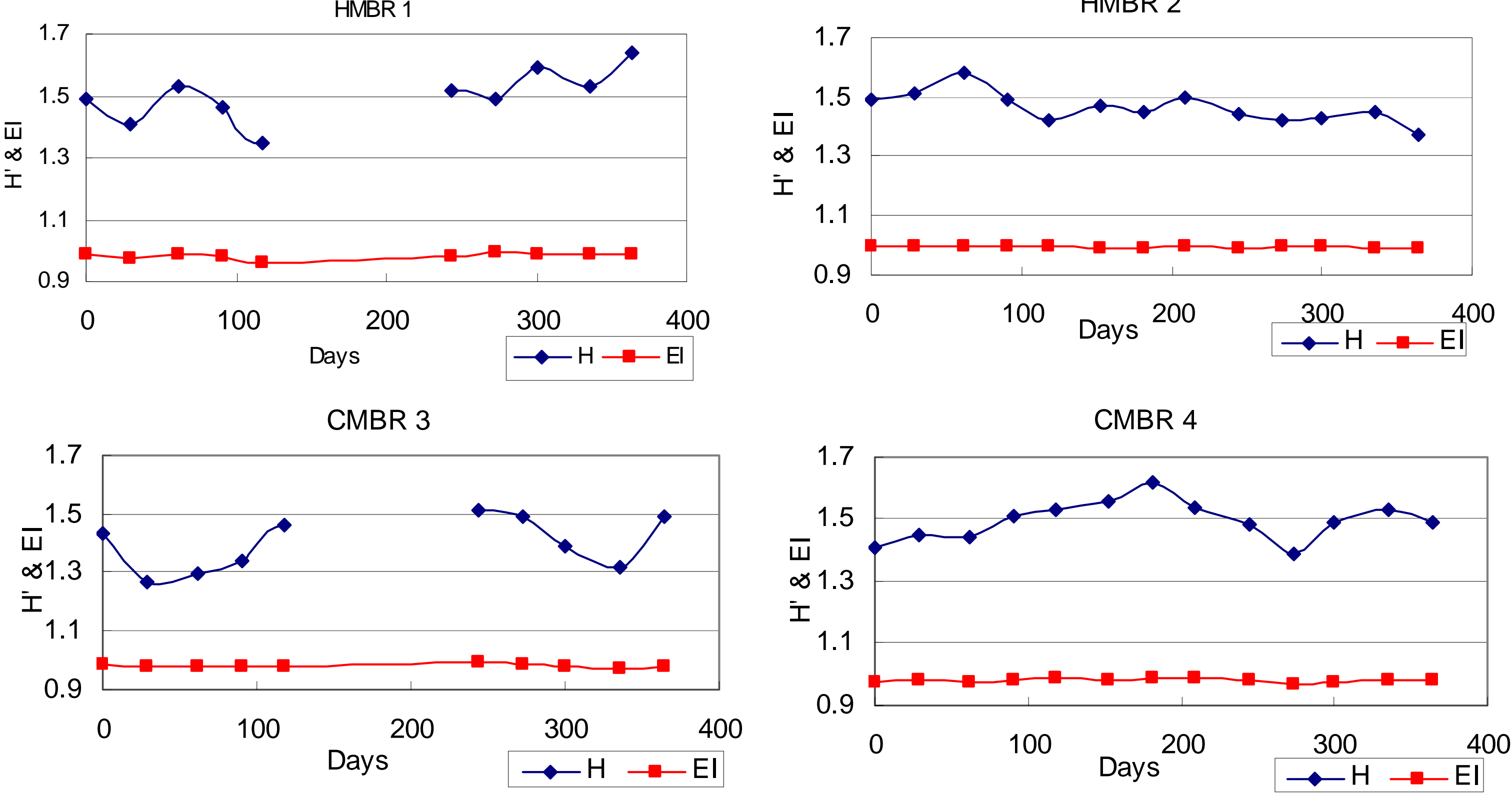
HMBR 2 (5%)



CMBR 3



CMBR 4 (25%)



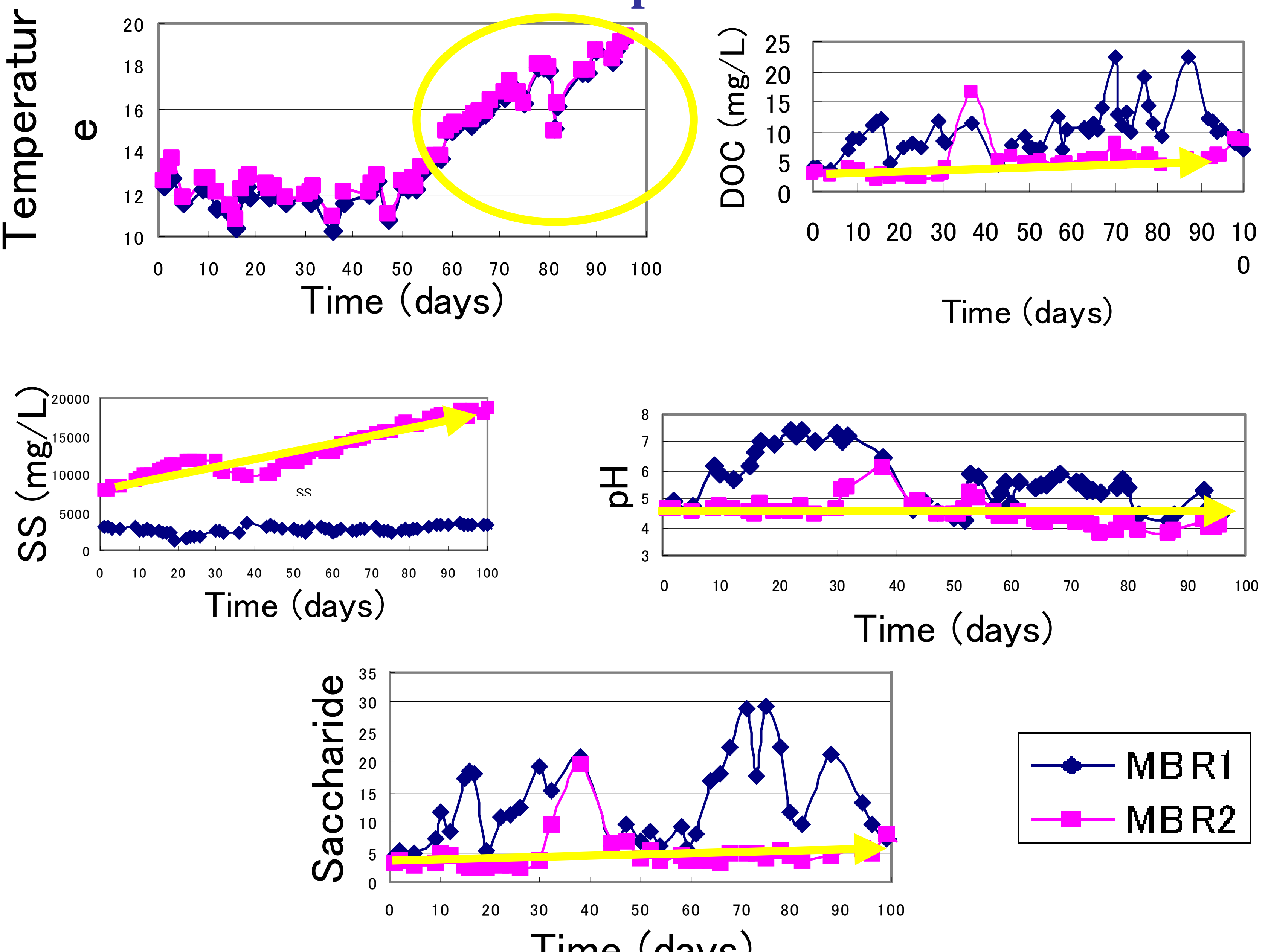
HMBR 1
HMBR 2
CMBR 3
CMBR 4
H' & EI
Days
H
EI
1.7
1.5
1.3
1.1
0.9
0
100
200
300
400

Operational parameters of the laboratory plants

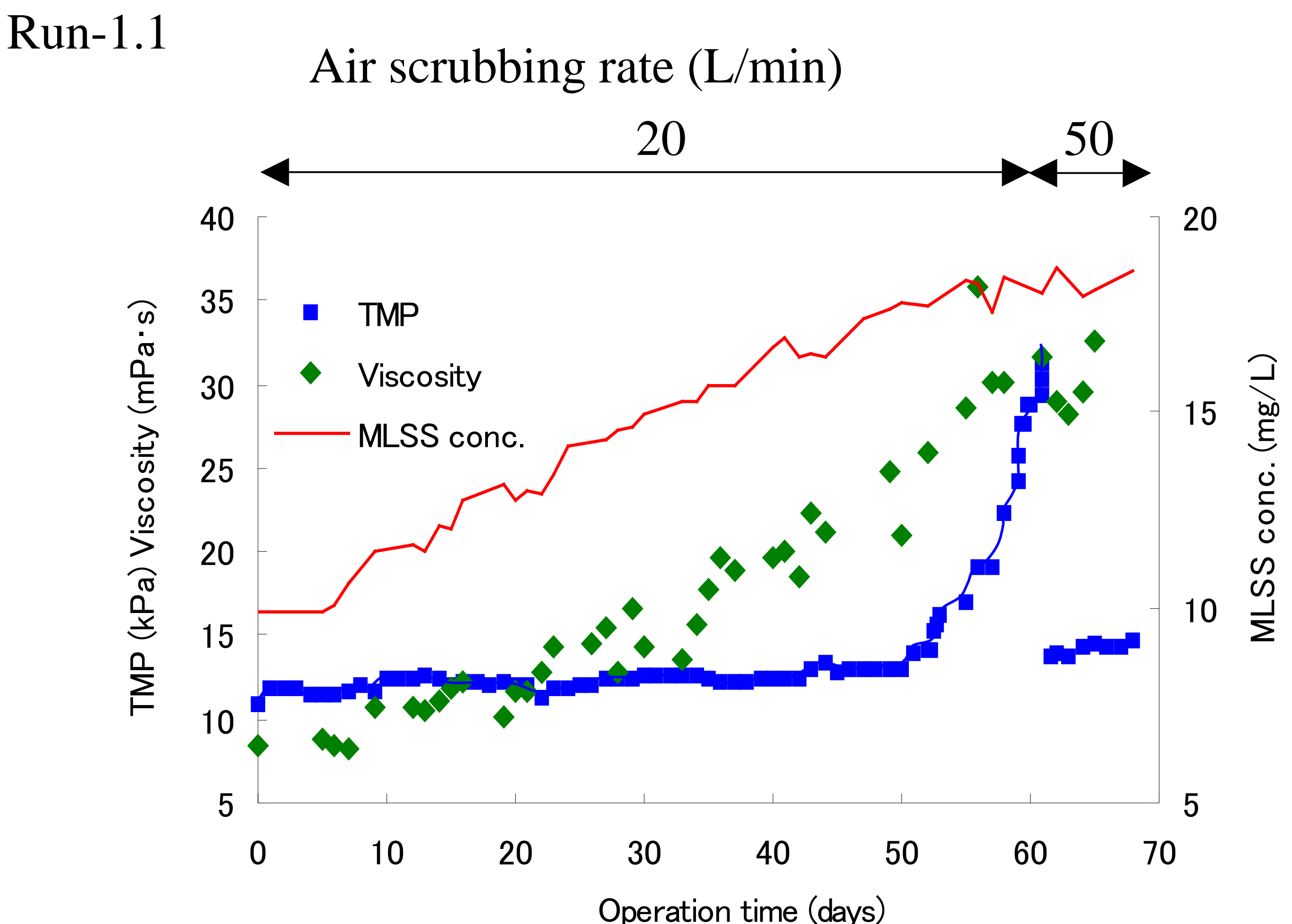
Parameter	MBR
Volume (l)	180
Pore size (μm)	0.4
Material	Hydrophilic polyethylene
Filtration area (m^2)	3
HRT (h)	First period: 5.3; Second period: 4.5 - 6
SRT (days)	H_1 = 45; H_2 = 77; C_1 = 20; C_2 = 38

- Pilot scale plant
- Continuous flow
- Fed with domestic wastewater

Reactor performance data



Variation of TMP in hybrid MBR



In high MLSS conc. and viscosity of mixed liquor, the air-scrubbing is required.

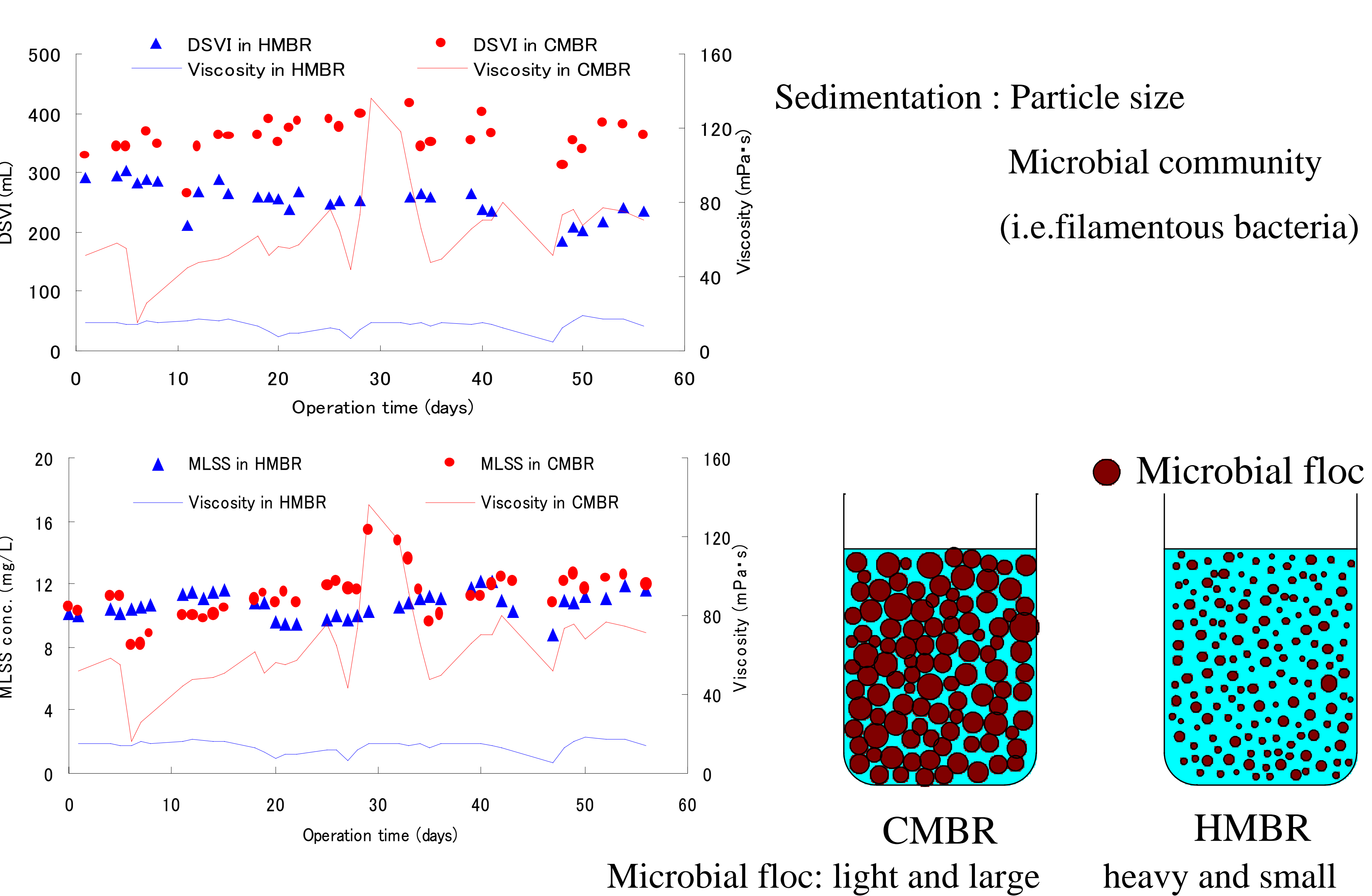
Variation of TMP in hybrid MBR of different MLSS conc.

Run-2

In Hybrid MBR 1 and 2, DOC conc. in mixed liquor was less than 5 mg/L.

Membrane permeability in high MLSS conc. is much higher than that in low MLSS conc..

Diluted Sludge volume index (DSVI)



	AS	H1	H2	C1	C2
EUB 338/DAPI *	80.52 ±22.1	61.21 ±15.3	38.96 ±15.7	61.16 ±17.5	41.39 ±10.8
ATP analysis	-	1047 ±204	938 ±232	2440 ±257	1769 ±333
EUB 338III/DAPI*	7.27 ±6.1	4.19 ±1.9	5.29 ±2.4	12.17 ±7.8	7.44 ±4.2

* For each sample, at least 20 different randomly chosen microscopic fields and a minimum of 1000 DAPI-stained cells were enumerated without counting the flocs and consequently, the filamentous bacteria within them. This could be introducing some errors in the AS and conventional MBRs.

EUB 338: members of the domain Bacteria

EUB 338 III: members of the phyla Verrucomicrobia

WWTPs and laboratory reactors									
	1	2	3	4	5	6	7	8	Average
No clones	62	94	96	97	92	51	92	150	92
No OTUs	25	53	33	69	75	30	50	16	44
eOTUs**	32	83	42	144	268	59	109	17	94
Coverage (%)”	77	64	78	48	28	51	46	93	61
Proteobacteria									
α	3 (1)	26 (15)	5 (4)	13 (8)	17 (15)	16 (8)	4 (3)	5 (1)	11
β	52 (9)	31 (99	51 (11)	33 (20)	25 (13)	8 (2)	17 (8)	14 (1)	29
γ	18 (17)		22 (6)	8 (4)	10 (9)	8 (3)	5 (2)	7 (2)	10
δ		2 (2)	4 (4)	3 (3)	1 (1)	4 (2)	3 (3)	1 (1)	2
ε	15 (1)		1 (1)	2 (2)	7 (5)	4 (2)			4
Bacteroidetes			2 (2)	5 (5)	13 (9)	6 (1)	39 (17)	50 (7)	14
Acidobacteria		5 (3)	5 (2)	3 (2)	7 (5)	2 (1)	9 (6)		4
Firmicutes	10 (5)	1 (1)		1 (1)				5 (1)	2
Actinobacteria		1 (1)	1 (1)	4 (3)	2 (2)	37 (3)	4 (2)	9 (2)	7
Nitrospira		2 (1)	8 (2)	3 (3)	2 (2)		3 (1)		2
Verrucomicrobia	2 (1)	3 (2)		1 (1)	1 (1)		4 (3)		2
Planctomycetes		12 (10)		13 (9).	9 (8)	8 (4)	3 (2)		6
Chorobi		1 (1)				2 (1)	3 (2)		1
Choroflexi	2 (1)	16 (8)		4 (3)	3 (3)	6 (3)			4
Fibrobacteres								9 (1)	1
Fusobacteria				3 (2)	2 (2)				1
OP11							1 (1)		0
Unaffiliated				3 (3)					0

FISH probes

Major groups of bacteria

Presumable polyphosphate accumulating organisms (PAOs)

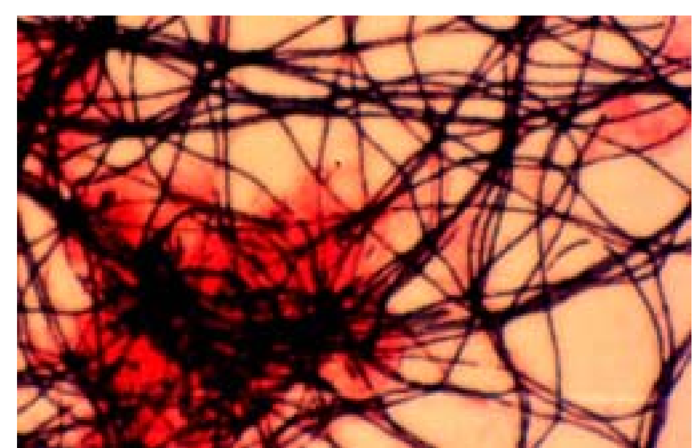
Filamentous bact.

Ammonium oxidizing bacteria

Probe	Specificity
EUB 338	Domain bacteria
ALF1b	a-Proteobacteria
BET42a	b-Proteobacteria
BONE23a	b1-Proteobacteria
GAM42a	g-Proteobacteria
CF319	Cytophaga-Flavobacteria-cluster
HGC69a	Gram-positive bacteria with high GC content of DNA
GNSB941	Phylum GNSB and relatives
Pla46	Planctomycetales and relatives
Aca23a	Acinetobacter spp.
Actino1011	Ebpr 19 and 20 (Tetrasphaera japonica, AF125092)
MP2	Microlunatus phosphorus
PAO846	PAO-Cluster (Rodocyclus spp.)
Hhy23a	Haliscomenobacter spp.
MNP1	Nocardioform actinomycetes
Sna23a	Sphaerotilus spp.
21N23a	Eikelboom Type 021N
Nso1225	Ammonium oxidizing b-Proteobacteria
Nsv443	Nitrospira spp.
NEU	Halophil and halotolerant members of the genus Nitrosomonas

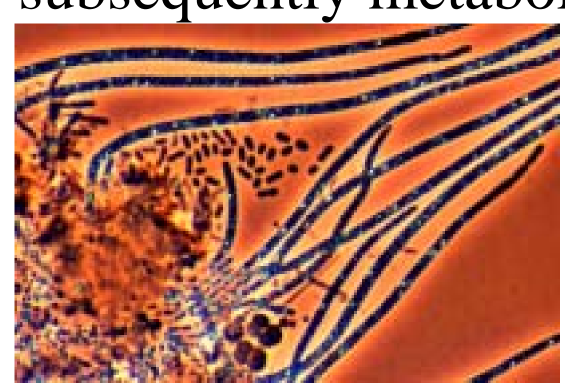
Filamentous bacteria

- ✓ Floc formation
- ✖ Foam formation
- ✖ Bulking

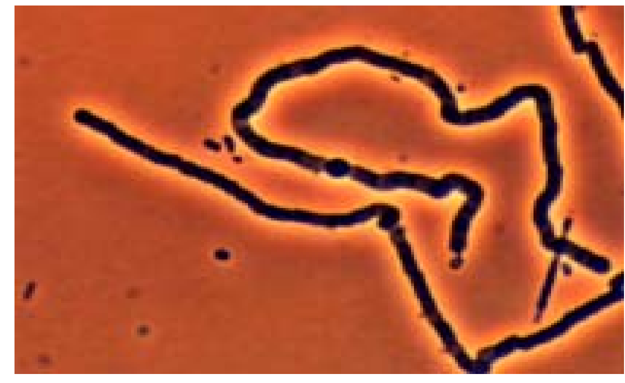


Microthrix parvicella: causative agent of the worldwide foaming and bulking problems in WWTPs with nutrient removal

MAR analyses reveal that this bacteria is able to take up and store long-chain fatty acids under anaerobic conditions and subsequently metabolize them under aerobic conditions.



Thiothrix spp.



Nostocoida limicola

Chloroflexi (Green non-sulfur bacteria)

Chloroflexi I

- Recently undergone significant expansion due to the addition of many environmental clone sequences.

- The environmental clones largely comes from pollutant-contaminated habitats, while two were isolated recently from an UASB reactor and from a hot spring sulfur-turf in Japan (Sekiguchi *et al*., 2001 and 2003).

- The majority of activated sludge clones were generated from a full-scale activated sludge biomass (Jurestchlo et al., 2002; Snadir et al, 1997)

Chloroflexi II

- Contain the well-known tetrachloroethene dechlorinator "*Dehalococcoides ethenogenes*"

Chloroflexi III

- Contain most of the pure-cultured representatives of Chloroflexi.

- It best known from hot springs and hypersaline isolates or clones but does contain "*Herpetosiphon* spp." obtained from full-scale activated sludge.

Chloroflexi VI

- Composed of clone sequences from marine and lake-water environments.

Chloroflexi in activated sludge

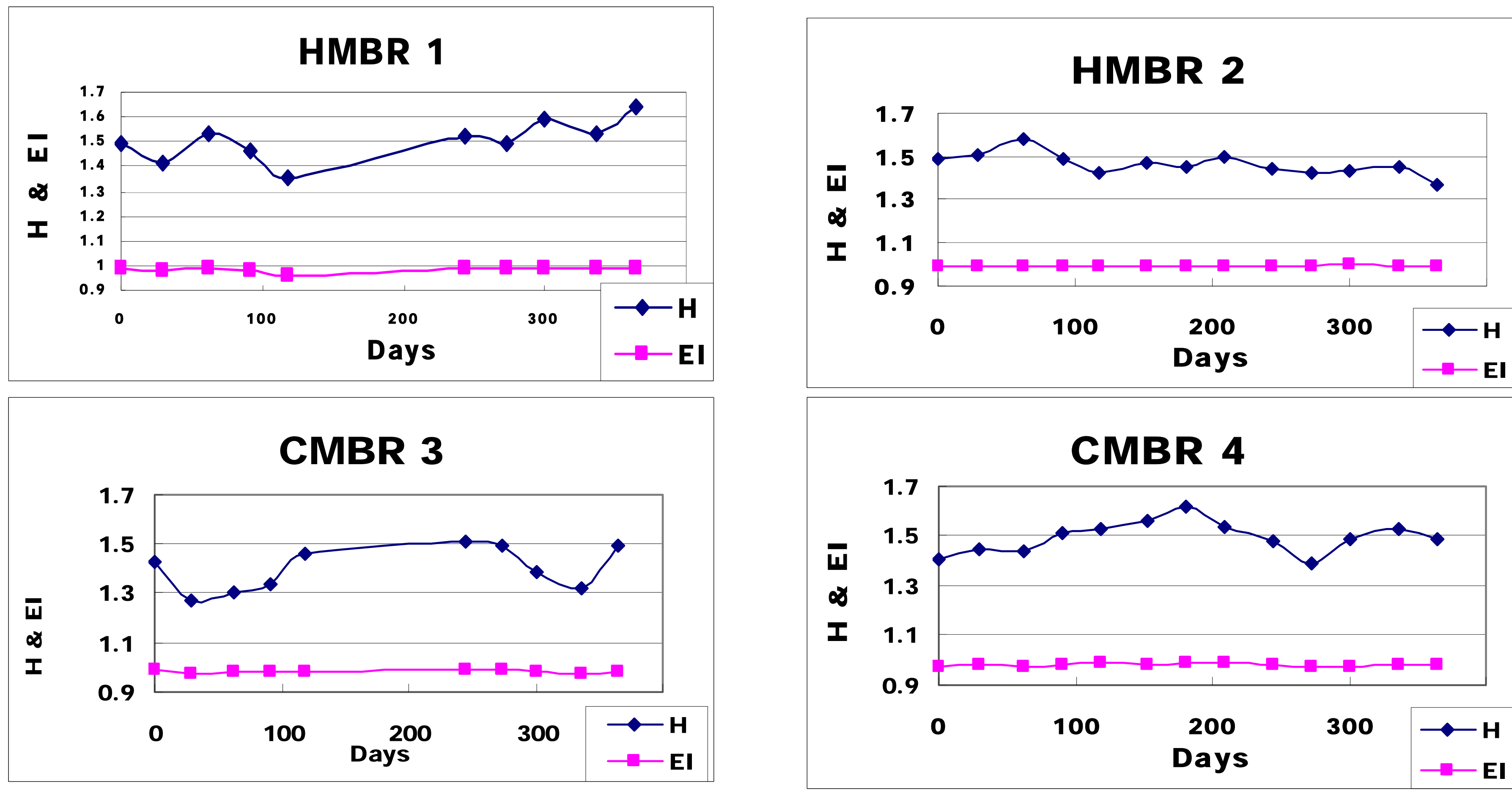
- Different studies have revealed a great biodiversity among Chloroflexi in other natural habitats than previously suspected. (Nübel *et al*., 2001)

- Application of 16S rRNA targeted probes designed against members of the Chloroflexi (Björnsson *et al*., 2002) to activated sludge biomass samples suggest that cells of this phylum are present there in large numbers, even though their role is not yet known.

- The incidence and importance of *Chloroflexi* in activated sludge is not known, although filaments of an *Herpetosiphon* sp. have been isolated and cultured earlier from bulking sludge (Bradford *et al*., 1996)

Much more needs to be learned about their taxonomic diversity and ecology before their environmental importance can be understood.

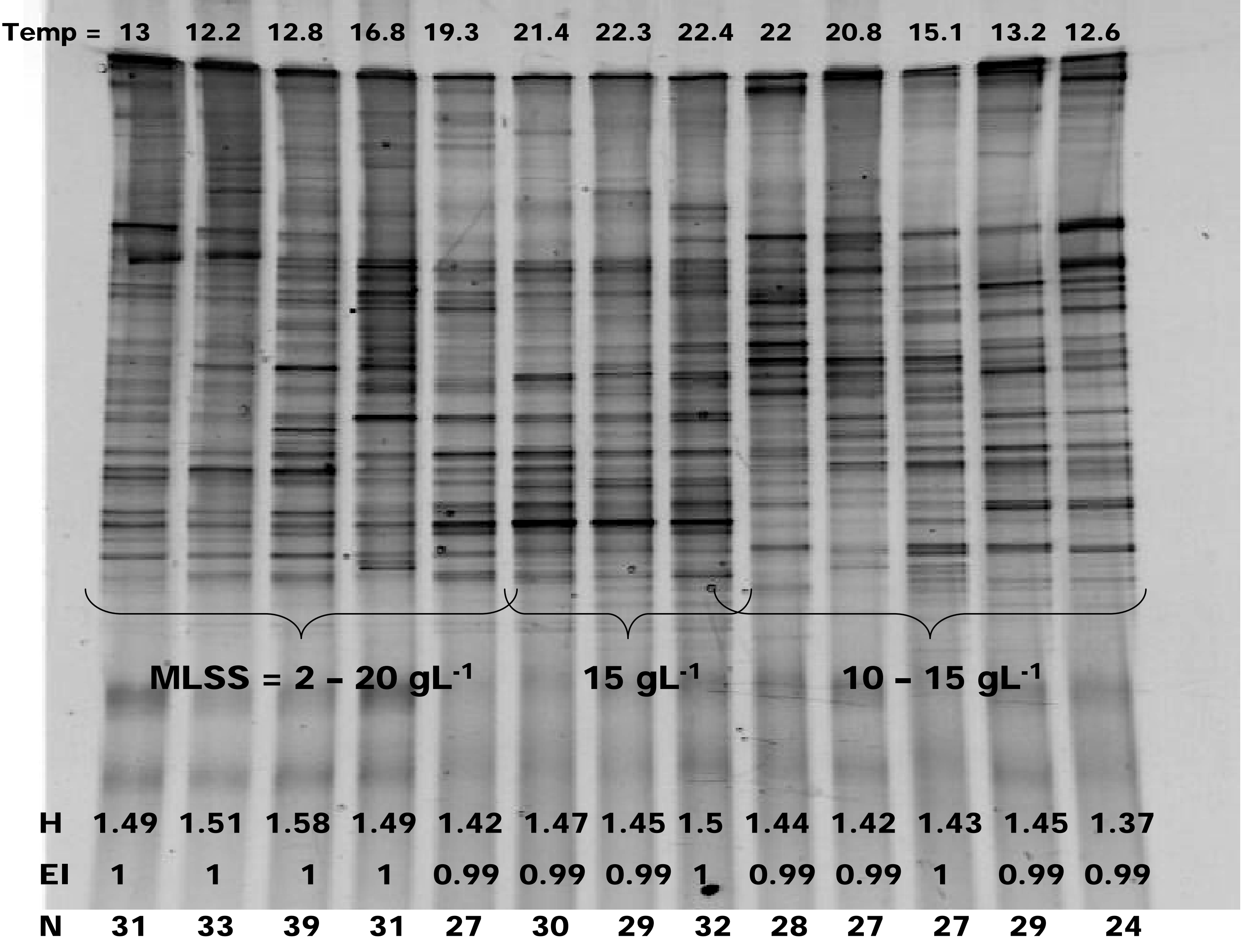
Shannon diversity index (H) & Equitability index (EI)



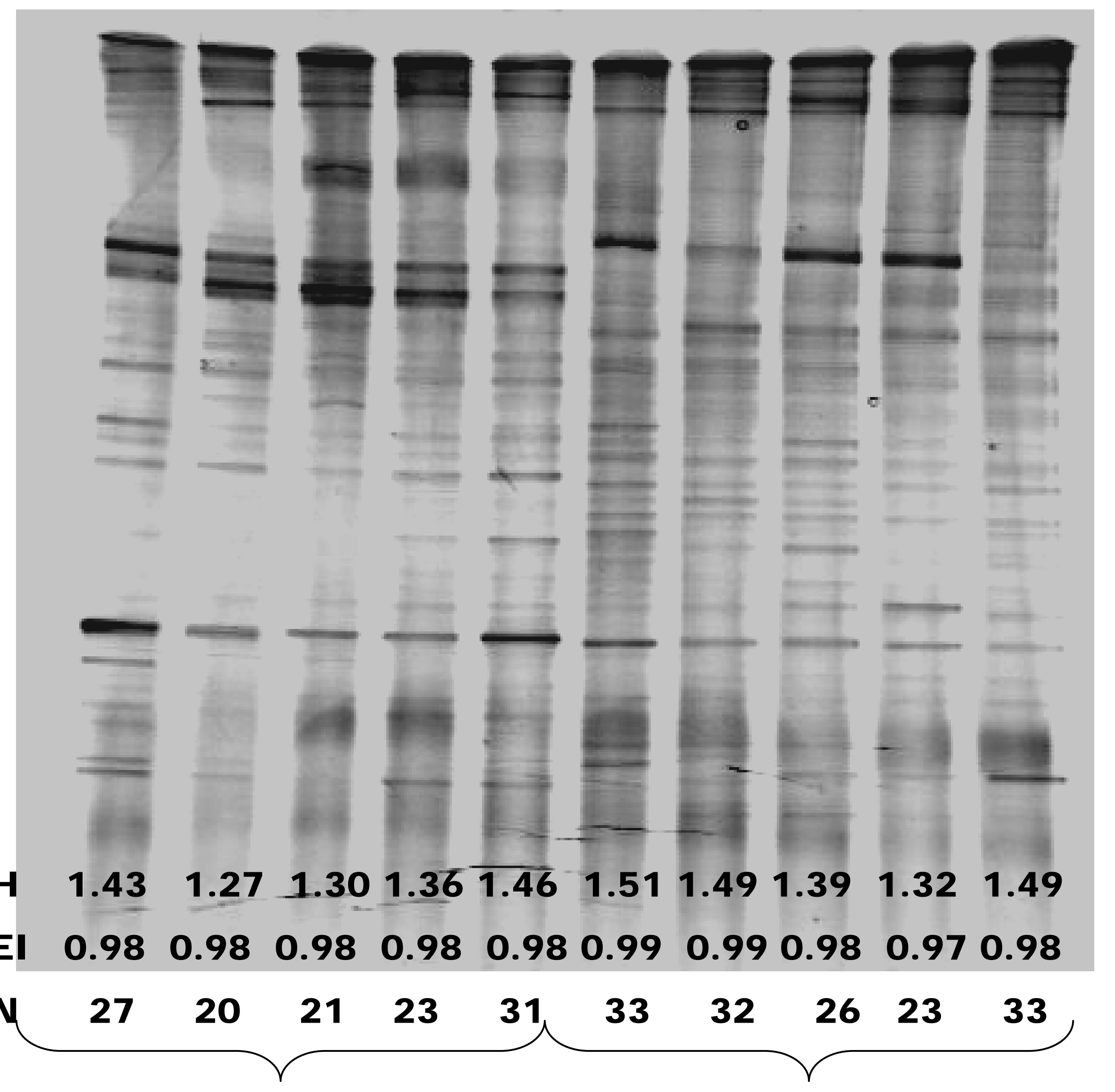
HMBR 2, the most functionally stable MBR showed the highest and uniform value of the Shannon diversity index (1.51 to 1.42) and Equitability index (0.99 – 1) during a stable operation but a little decrease in the value was observed when the **operation became unstable.**

Stable operation = high community diversity and even distribution of species

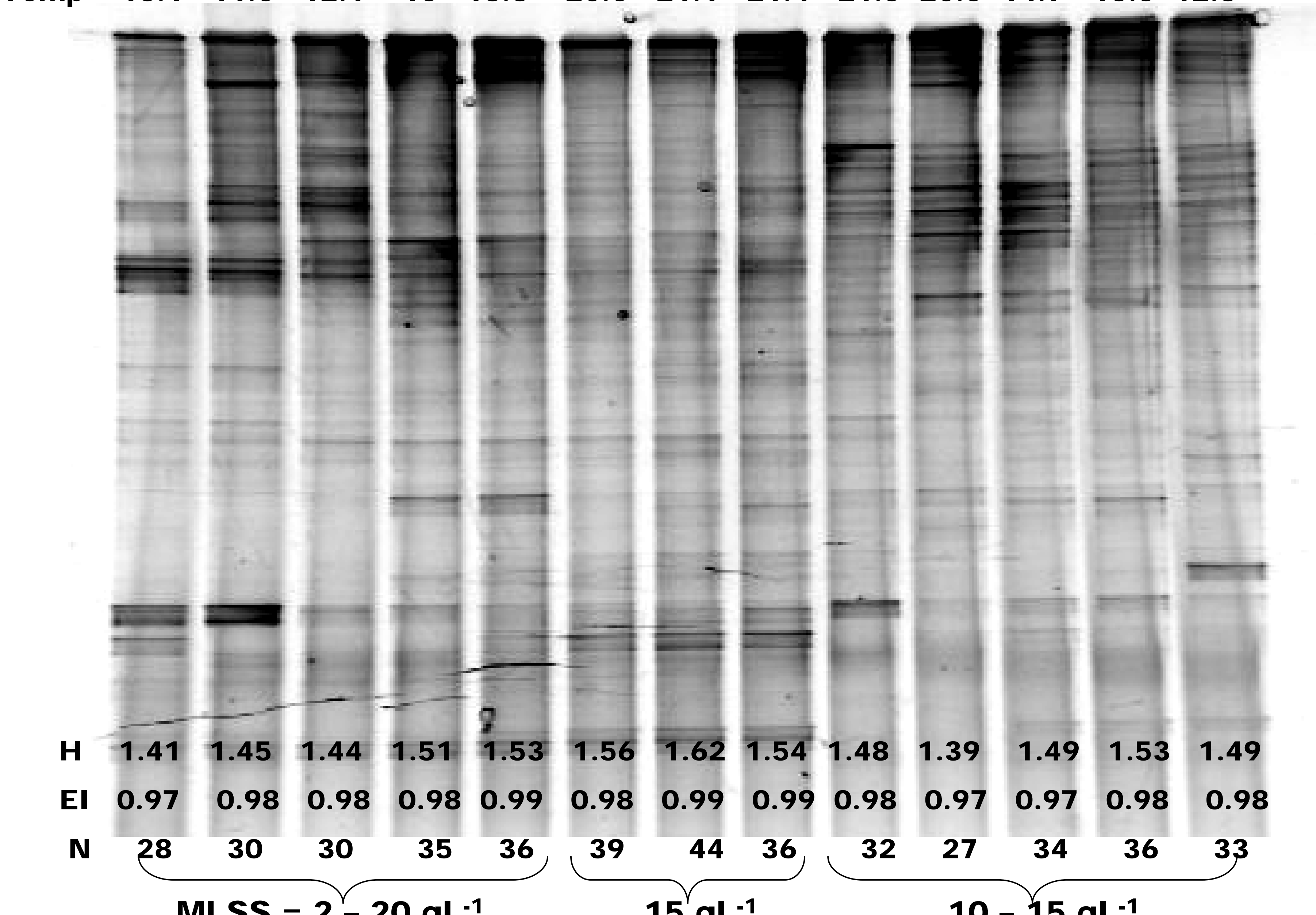
1/17 2/15 3/20 4/18 5/15 6/18 7/17 8/14 9/18 10/17 11/13 12/19 1/16



HMBR 2 357F-GC/518R



CMBR 3
357F-GC/518R
1/17 2/15 3/20 418 5/15 9/18 10/17 11/13 12/19 1/16
Temp = 12.3 11.9 12.6 16.8 19.4 22 20.8 15.1 13.2 12.6
H 1.43 1.27 1.30 1.36 1.46 1.51 1.49 1.39 1.32 1.49
EI 0.98 0.98 0.98 0.98 0.98 0.99 0.99 0.98 0.97 0.98
N 27 20 21 23 31 33 32 26 23 33
MLSS = 2 – 4 gL-1
MLSS = 5 – 7 gL-1



CMBR 4
357F-GC/518R
1/17 2/15 3/20 4/18 5/15 6/18 7/17 8/14 9/18 10/17 11/13 12/19 1/16
Temp = 13.1 11.6 12.1 16 18.8 20.6 21.1 21.4 21.5 20.5 14.7 13.5 12.8
H 1.41 1.45 1.44 1.51 1.53 1.56 1.62 1.54 1.48 1.39 1.49 1.53 1.49
EI 0.97 0.98 0.98 0.98 0.99 0.98 0.99 0.99 0.98 0.97 0.97 0.98 0.98
N 28 30 30 35 36 39 44 36 32 27 34 36 33
MLSS = 2 – 20 gL-1
15 gL-1
10 – 15 gL-1