

16S rRNA PCR-DGGE BASED BACTERIAL COMMUNITY
STRUCTURE IN ACTIVATED SLUDGE SYSTEMS

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Abstract

We studied bacterial community structure in three different sewage treatment plants (STPs), namely STP 1, 2 and 3, in Klang Valley. STP 1, 2 and 3 were located in residential, commercial and light-industrial areas, respectively. Cu and Pb pollution detected in STP 3 was shown to affect its sludge production and biomass. Investigation by 16S rRNA PCR-DGGE demonstrated that bacterial community structures in the three STPs were different; and heavy metal resistant bacteria were predominant at STP 3. In order to determine the effects of heavy metal pollution on bacterial community structures, we used the canonical correspondence analysis, and showed that there were two distinct groups of bacteria. The ecological distance of one group reflected the effects of both Cu and Pb pollution whereas another group mirrored the sludge volume. In this study, we showed that heavy metal pollution affected the bacterial community structure in the aeration tank. We also showed by cluster analysis of the DGGE banding patterns that the bacterial community in each STP was relatively stable. Although the stable bacterial community structure suggested acclimatization, the bacterial community in the impacted STP was not conducive for floc development, and therefore affected its efficiency.

Abstrak

Kita mengkaji struktur komuniti bakteria dalam tiga loji rawatan kumbahan yang berbeza (STP 1, 2 dan 3) di Lembah Klang. STP 1, 2 dan 3 masing-masing terletak di kawasan kediaman, komersial dan perindustrian ringan. Pencemaran Cu dan Pb di STP 3 menunjukkan penjejasan pengeluaran enapcemar dan biomas. Penyiasatan 16S rRNA-DGGE menunjukkan bahawa struktur komuniti bakteria di dalam tiga STP adalah berbeza dan terdapat dominasi bakteria logam berat di STP 3. Untuk menentukan kesan pencemaran logam berat terhadap struktur komuniti bakteria, kami menggunakan “canonical correspondence analysis” dan menunjukkan bahawa terdapat dua kumpulan bakteria yang berbeza. Jarak ekologi salah satu kumpulan bakteria mencerminkan kesan pencemaran kedua-dua Cu dan Pb, manakala satu kumpulan lagi mencerminkan isipadu enapcemar. Dalam kajian ini, kami menunjukkan bahawa pencemaran logam berat menjejaskan struktur komuniti bakteria di dalam tangki pengudaraan STP-STP. Dalam analisis kelompok corak jalur DGGE, kami juga menunjukkan bahawa komuniti bakteria di dalam setiap STP relatif adalah stabil. Walaupun struktur komuniti bakteria yang stabil mencadangkan aklimatisasi, tetapi komuniti bakteria di STP berkenaan tidak konduktif untuk pertumbuhan floc, dan dengan ini ia menjejaskan kecekapan proses.

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List of Symbols and Abbreviations

Symbol and unit

δ	:	delta
γ	:	gamma
β	:	beta
α	:	alpha
$^{\circ}\text{C}$:	degree Celsius
$^{\circ}$:	degree
\pm	:	plus minus
$>$:	more than
$<$:	less than
'	:	minute
%	:	percent
$\text{m}^3\text{ha}^{-1}\text{d}^{-1}$:	cubic meter per hectare per day
μm	:	micrometer
$\mu\text{m mg}^{-1}$:	micrometer per milligram
g	:	relative centrifugal force
g	:	gram
$g\ \text{l}^{-1}$:	gram per liter
M	:	molar
$\text{m}^3\ \text{day}^{-1}$:	cubic meter per day
$\text{mg}\ \text{l}^{-1}$:	milligram per liter
$\text{mg}\ \text{l}^{-1}\ \text{hr}^{-1}$:	milligram per liter per hour
ml	:	milliliter
$\text{ml}\ \text{l}^{-1}$:	milliliter per liter

mm	:	milliliter
mM	:	millimolar
mm ²	:	square millimeter
ng μl ⁻¹	:	nanogram per microliter
V	:	volume
V cm ⁻¹	:	volt per centimeter
v/v	:	volume per volume
μl	:	microliter

Chemical substance and formula

Ag ₂ SO ₄	:	silver sulfate
As	:	arsenic
CaCl ₂	:	calcium chloride
Cd	:	cadmium
Cr	:	chromium
Cu	:	copper
FeCl ₃	:	iron (III) chloride
H ₂ O	:	dihydrogen oxide
H ₂ SO ₄	:	sulfuric acid
Hg	:	mercury
K ₂ HPO ₄	:	dipotassium phosphate
KH ₂ PO ₄	:	potassium dihydrogen phosphate
MgCl ₂	:	magnesium chloride
MgSO ₄	:	magnesium sulfate
Na ₂ HPO ₄	:	sodium phosphate
NaCl	:	sodium chloride

NH ₃	:	ammonia
NH ₄ Cl	:	ammonium chloride
NO ₂ ⁻	:	nitrite
NO ₃ ⁻	:	nitrate
Pb	:	lead
PO ₄ ³⁻	:	phosphate
SDS	:	sodium dodecyl sulfate

Abbreviation

AAS	:	atomic absorption spectrophotometry
ANOSIM	:	analysis of similarities
ANOVA	:	analysis of variance
APHA	:	American Public Health Association
BLAST	:	Basic Local Alignment Search Tool
BOD	:	biological oxygen demand
bp	:	base pair
CCA	:	canonical correspondence analysis
COD	:	chemical oxygen demand
CVAA	:	cold-vapor atomic absorption
<i>df</i>	:	degree of freedom
DFA	:	dissolved air flotation
DGGE	:	denaturing gradient gel electrophoresis
DNA	:	deoxyribonucleic acid
dNTPs	:	deoxynucleotide triphosphates
DOUR	:	dissolved oxygen uptake rate
E	:	east

e.g.	:	exempli gratia of example given
<i>et al</i>	:	et alia
<i>f</i>	:	normality of sodium thiosulfate solution
F	:	frequency
FAS	:	ferrous ammonium sulfate solution
GF/C	:	glass fiber cartridge
i.e.	:	id est or that is
ICP-AES	:	inductive coupled plasma-atomic emission spectrometry
MLSS	:	mixed liquor suspended solids
MLVSS	:	mixed liquor volatile suspended solids
MUSCLE	:	multiple sequence comparison by log- expectation
<i>n</i>	:	sample size
n	:	number of time occur
N	:	north
NCBI	:	National Center for Biotechnology Information
OTU	:	operational taxonomic unit
PAST	:	Palaeontological Statistics
PCR	:	polymerase chain reaction
PE	:	population equivalent
PLFA	:	phospholipids fatty acid
R^2	:	coefficient of determination
rDNA	:	ribosomal deoxyribonucleic acid
RDP	:	Ribosomal Database Project
RNA	:	ribonucleic acid
rRNA	:	ribosomal ribonucleic acid
SIMPER	:	similarity percentage

SSV	:	settled sludge volume
STPs	:	sewage treatment plants
SV	:	sludge volume
SVI	:	sludge volume index
TAE	:	tris-acetate-ethylenediaminetetraacetic acid
TBE	:	tris-borate-ethylenediaminetetraacetic acid
TE	:	tris-ethylenediaminetetraacetic acid
TEMED	:	N,N,N',N'-tetramethylethylenediamine
TGGE	:	temperature gradient gel electrophoresis
TRFLP	:	terminal restriction fragment length polymorphism
TRFs	:	terminal restriction fragments
TSS	:	total suspended solids
USEPA	:	United State Environmental Protection Agency
UV	:	ultraviolet