Electronic Supplementary Information for

Cotreatment of landfill leachate and high-nitrate wastewater using SBRs: Evaluation of denitrification performance and microbial analysis

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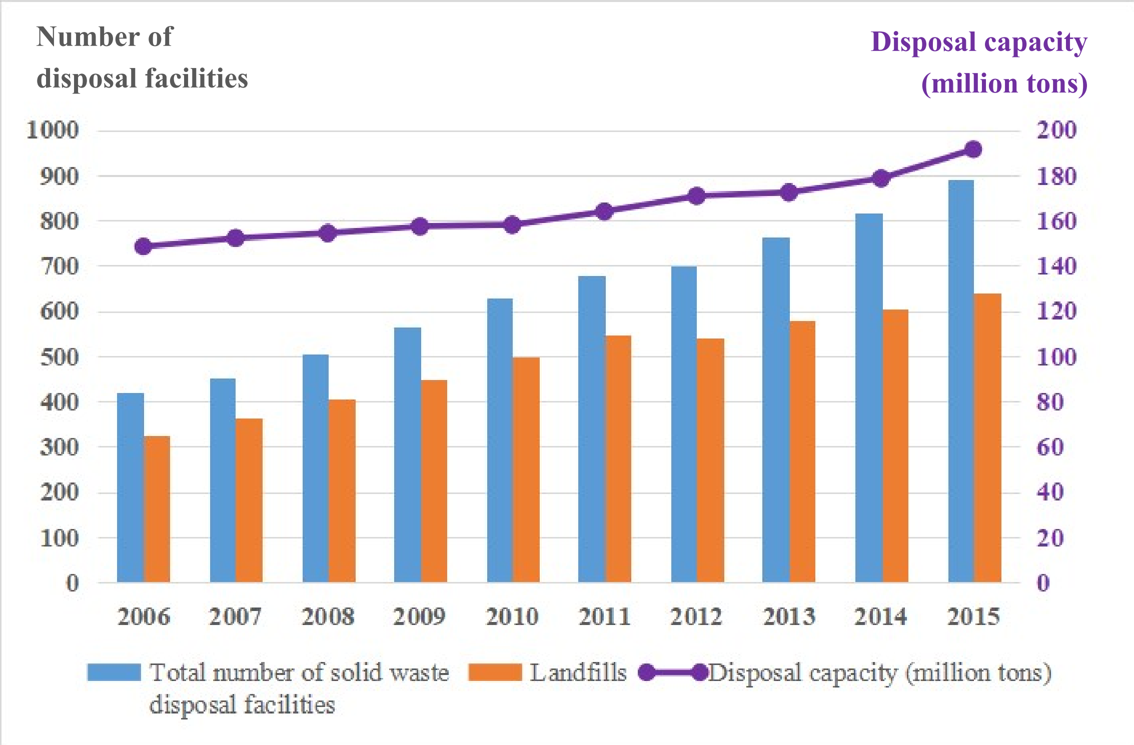
(A)



(B)

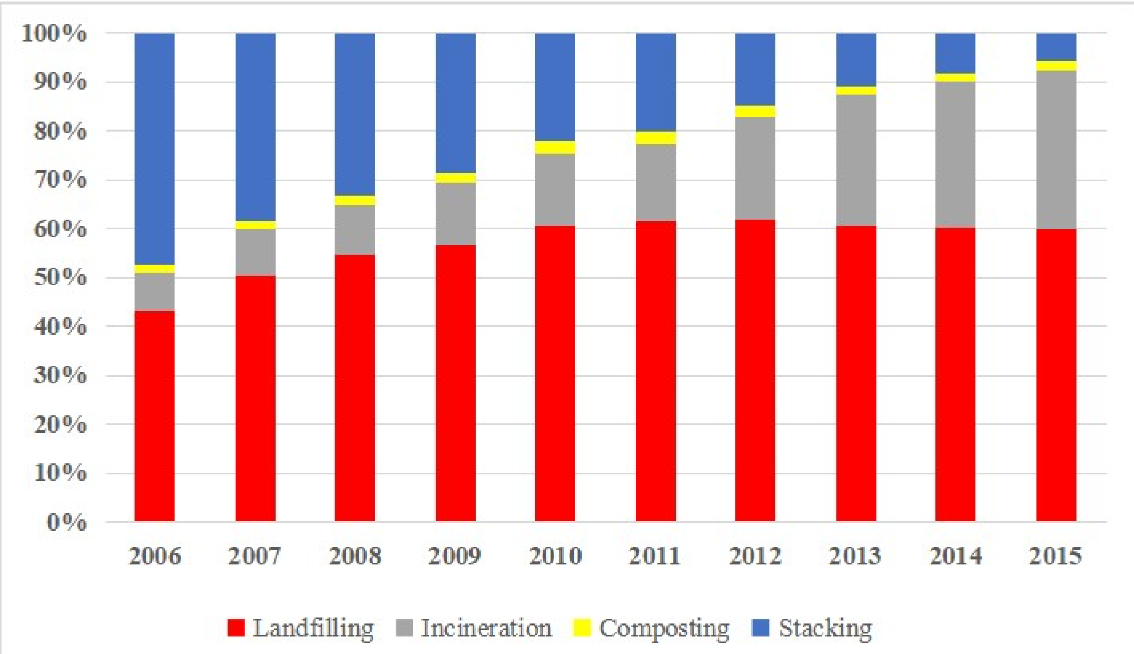
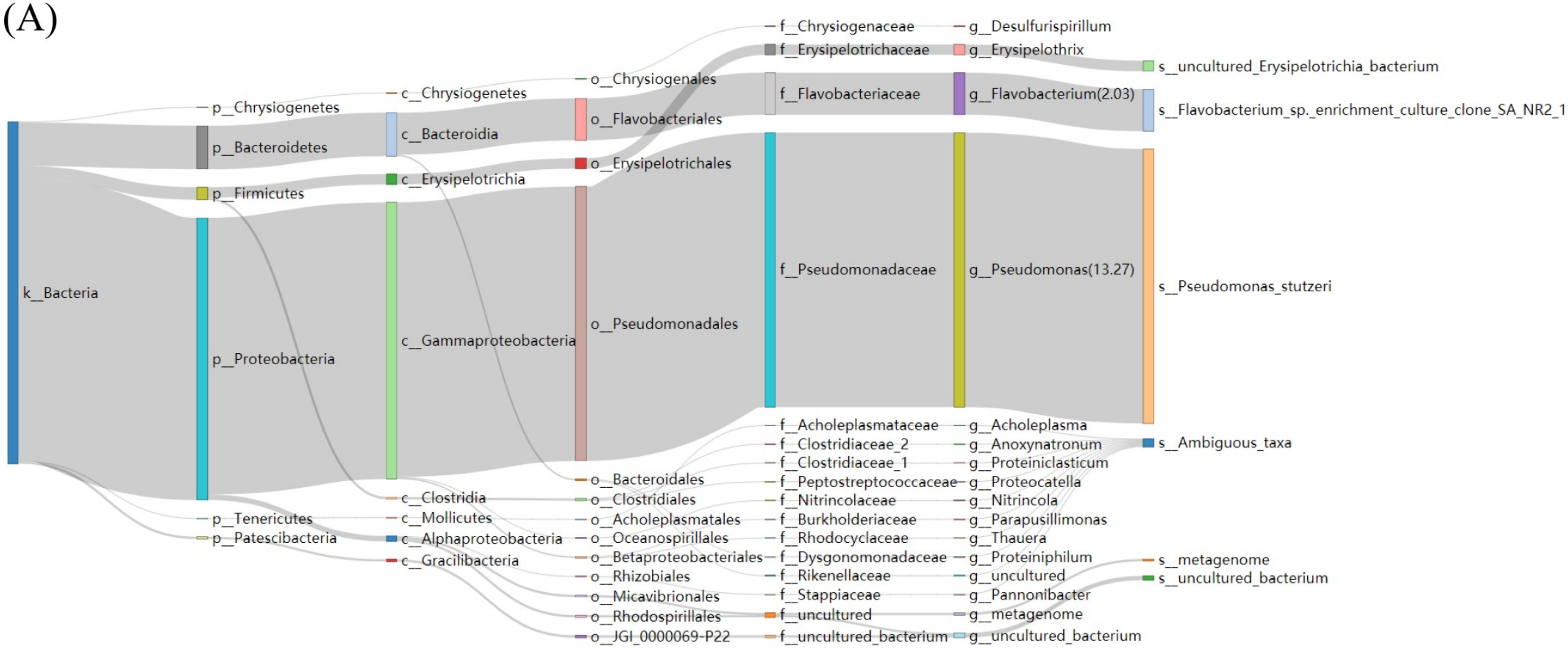


Fig. S1 Disposal methods, capacity and acilities of municipal solid waste in China from 2006 to 2015. Data from the Professional Committee of Urban Domestic Refuse Treatment of CAEPI.

(A)



k__Bacteria
p__Chrysiogenetes
p__Bacteroidetes
p__Firmicutes
p__Proteobacteria
p__Tenericutes
p__Patescibacteria
c__Chrysiogenetes
c__Bacteroidia
c__Erysipelotrichia
c__Gammaproteobacteria
c__Clostridia
c__Mollicutes
c__Alphaproteobacteria
c__Gracilibacteria
o__Chrysiogenales
o__Flavobacteriales
o__Erysipelotrichales
o__Pseudomonadales
o__Bacteroidales
o__Clostridiales
o__Acholeplasmatales
o__Oceanospirillales
o__Betaproteobacteriales
o__Rhizobiales
o__Micavibrionales
o__Rhodospirillales
o__JGI_0000069-P22
f__Chrysiogenaceae
f__Erysipelotrichaceae
f__Flavobacteriaceae
f__Pseudomonadaceae
f__Acholeplasmataceae
f__Clostridiaceae_2
f__Clostridiaceae_1
f__Peptostreptococcaceae
f__Nitrincolaceae
f__Burkholderiaceae
f__Rhodocyclaceae
f__Dysgonomonadaceae
f__Rikenellaceae
f__Stappiaceae
f__uncultured
f__uncultured_bacterium
g__Desulfurispirillum
g__Erysipelothrix
g__Flavobacterium(2.03)
g__Pseudomonas(13.27)
g__Acholeplasma
g__Anoxynatronum
g__Proteiniclasticum
g__Proteocatella
g__Nitrincola
g__Parapusillimonas
g__Thauera
g__Proteiniphilum
g__uncultured
g__Pannonibacter
g__metagenome
g__uncultured_bacterium
s__uncultured_Erysipelotrichia_bacterium
s__Flavobacterium_sp._enrichment_culture_clone_SA_NR2_1
s__Pseudomonas_stutzeri
s__Ambiguous_taxa
s__metagenome
s__uncultured_bacterium

(B)

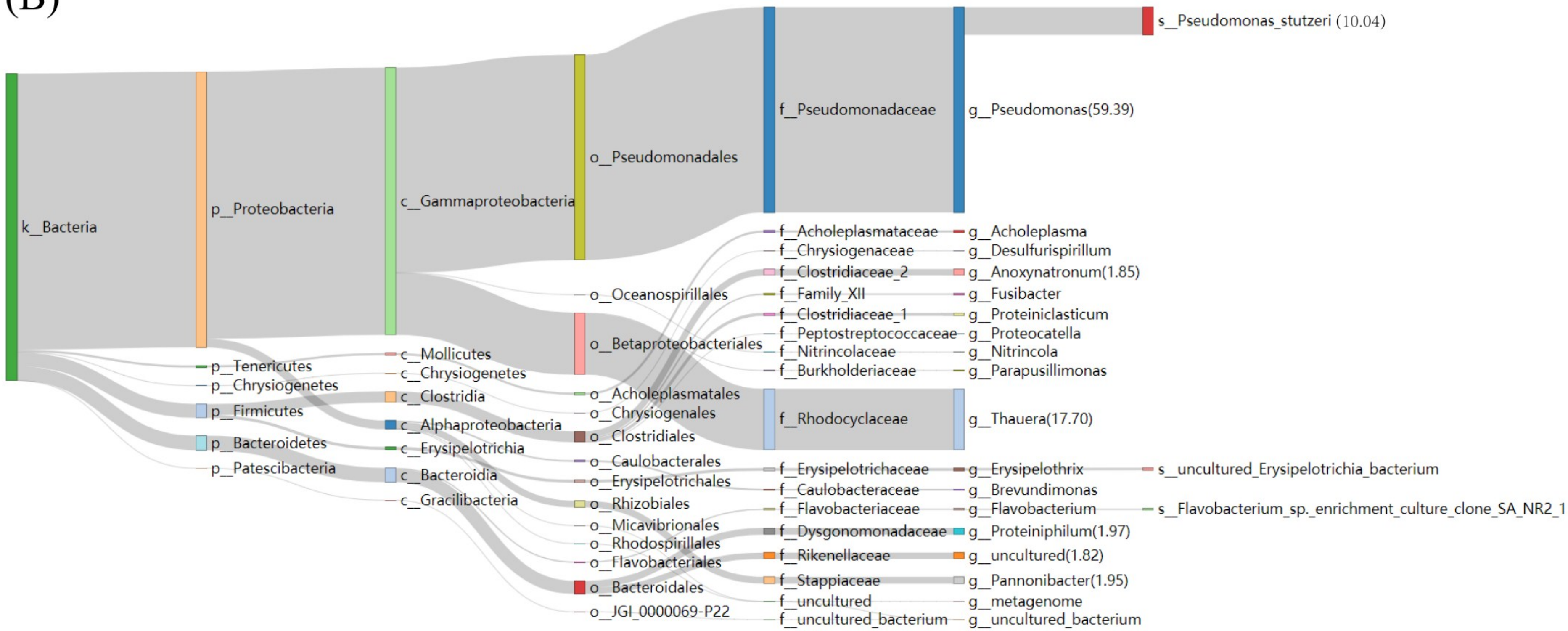


Fig.2 Evolutionary tree of microbial population. Different colors represent different annotations, the size of the bar graph represents the abundance of the species, and the connection represents the subordinate relationship (aggregation from low level). **(A)**, R1 system. **(B)**, R0 system.

Table S1 Physicochemical characteristics of raw landfill leachate and pretreated landfill leachate.

Parameter	Abbr.	Raw landfill leachate			Pretreated landfill leachate		
		Max	Min	Mean	Max	Min	Mean
pH	--	9.1	7.6	8.2	8.9	7.4	7.9
Suspended solids	SS	1803	917	1360	1021	207	614
Chemical oxygen demand	COD	12660	4420	5710	8400	3200	4610
5-day biochemical oxygen demand	BOD_5	3690	1020	1490	3460	950	1370
Ammonium nitrogen	NH+ 4-N	4190	1770	2480	586	164	275
Nitrate nitrogen	NO- 3-N	11.4	nd	5.3	18.0	nd	12.0
Nitrite nitrogen	NO- 2-N	5.2	nd	2.8	7.0	nd	6.0
Total phosphorus	TP	78.1	15.5	33.4	26.0	5.0	3.0
Total nitrogen	TN	5210	1950	2530	910	190	560
Copper ion	Cu^{2+}	0.22	0.017	0.039	0.16	0.011	0.028
Divalent zinc	Zn^{2+}	0.84	0.26	0.55	0.67	0.18	0.39
Calcium ion	Ca^{2+}	389	161	335	253	148	207
Magnesium ion	Mg^{2+}	54.7	19.5	31.8	30.5	11.7	20.5
Trivalent iron	Fe^{3+}	1.89	0.55	1.62	0.95	0.51	0.78
Hexavalent chromium	Cr^{6+}	0.659	0.017	0.102	0.35	0.024	0.085
Molybdenum	Mo	0.012	0.0031	0.0078	0.0099	0.0014	0.0039
Arsenic	As	0.1080	0.0075	0.0261	0.13	nd	0.0209
Mercury	Hg	0.00394	0.00015	0.00088	0.005	nd	0.00074
Cadmium	Cd	nd	nd	nd	nd	nd	nd
Lead	Pb	nd	nd	nd	nd	nd	nd
Chromium	Cr	0.98	0.17	0.56	1.3	0.2	0.41

Table S2 Components dosage of the synthetic high-nitrate wastewater fed to the SBR during domestication stage.

Condition	Reagents	Dosage ($g \cdot L^{-1}$)					
		0-5 cycle	6-12 cycle	13-18 cycle	19-23 cycle	24-30 cycle	31-46 cycle
Synthetic wastewater[a]	CH_3COONa	0.36	0.71	1.43	2.14	2.86	3.57
	KNO_3	0.083	0.16	0.33	0.49	0.65	0.81
	KH_2PO_4	0.004	0.009	0.017	0.025	0.037	0.04
	$MgSO_4$	0.015	0.015	0.025	0.025	0.03	0.03
	$CaCl_2$	0.03	0.03	0.05	0.05	0.06	0.06
Trace elements[b]	$FeCl_3 \cdot 6H_2O$	1.50	1.50	1.50	1.50	1.50	1.50
	$CuSO_4 \cdot 5H_2O$	0.03	0.03	0.03	0.03	0.03	0.03
	$ZnSO_4 \cdot 7H_2O$	0.12	0.12	0.12	0.12	0.12	0.12
	$CoCl_2 \cdot 6H_2O$	0.15	0.15	0.15	0.15	0.15	0.15
	EDTA	10.00	10.00	10.00	10.00	10.00	10.00
	KI	0.18	0.18	0.18	0.18	0.18	0.18
	$Na_2MoO_4 \cdot 2H_2O$	0.06	0.06	0.06	0.06	0.06	0.06
	H_3BO_3	0.15	0.15	0.15	0.15	0.15	0.15
	$MnCl_2 \cdot 4H_2O$	0.12	0.12	0.12	0.12	0.12	0.12

Note: a/b at a volume ratio of 1000:1

Table S3 Detection methods and instrument models of wastewater indicators.

Parameter	Methods	Manufacturer and model of the Instrument
SS	Gravimetry method 2540 [a]	XS105DU electronic analytical balance
COD	Colourimetric method 5220 D [a]	Hach COD reactor 45600- 00/Hach DR 2010 spectrophotometer
BOD_5	method 5210 B [a]	Hach BODTrakII respirometric apparatus
NH+ 4-N	Nessler Reagent Spectrophotometry	Shimadzu UV-160A spectrophotometer
NO- 3-N	method 4500 C - NO- 3 [a]	Shimadzu UV-160A spectrophotometer
NO- 2-N	method 4500 C - NO- 2 [a]	Shimadzu UV-160A spectrophotometer
TP	Ammonium molybdate spectrophotometry	Shimadzu UV-160A spectrophotometer
TN	Alkaline potassium persulfate digestion ultraviolet spectrophotometry	Shimadzu UV-160A spectrophotometer
Zn^{2+}	Ion chromatography	Thermo ICS-1100 Ion chromatograph
Cu^{2+}	Ion chromatography	Thermo ICS-1100 Ion chromatograph
Ca^{2+}	Ion chromatography	Thermo ICS-1100 Ion chromatograph
Mg^{2+}	Ion chromatography	Thermo ICS-1100 Ion chromatograph
Cr^{6+}	Ion chromatography	Thermo ICS-1100 Ion chromatograph
Fe^{3+}	Ion chromatography	Thermo ICS-1100 Ion chromatograph
Mo	Inductively Coupled Plasma Method	Agilent 7900 ICP-MS
As	Inductively Coupled Plasma Method	Agilent 7900 ICP-MS
Hg	Inductively Coupled Plasma Method	Agilent 7900 ICP-MS
Cd	Inductively Coupled Plasma Method	Agilent 7900 ICP-MS
Pb	Inductively Coupled Plasma Method	Agilent 7900 ICP-MS
Cr	Inductively Coupled Plasma Method	Agilent 7900 ICP-MS

[a] Standard Methods for the Examination of Water and Wastewater

Table S4 Primers and programs for PCR and quantitative real-time PCR of the five denitrifying functional genes and 16S rDNA in this study.

Target gens	Primers	Sequence (5'-3')	q-PCR (40 Cycles)		PCR (35 Cycles)			Fragment size (bp)	Ref.
			Annealing Temp /°C	Thermal profile	Denaturation	Annealing Temp/°C	Extension		
*nar*G	*nar*G-F	TAYGTSGGGCAGGARAAACTG	59.5	95°C/5min; 95°C/20s, 59.5°C/30s, 72°C/40s	95°C/5min	95°C/30s, 59.5°C/45s, 72°C/50s	72°C/7min	110	Lopez-Gutierrez et al.
	*nar*G-R	CGTAGAAGAAGCTGGTGCTGTT							
*nir*K	*nir*K-F	ATCATGGTSCTGCCGCG	62.5	95°C/3min; 95°C/40s, 62.5°C/40s, 72°C/60s	94°C/3min	94°C/30s, 62.5°C/30s, 720C/60s	72°C/10min	473	Hallin et al.
	*nir*K-R	GCCTCGATCAGRTTGTGGTT							
*nir*S	*nir*S-F	GTSAACGTSAAGGARACSGG	59	95°C/5min; 95°C/60s, 59°C/60s, 72°C/60s	94°C/3min	94°C/30s, 59°C/30s, 72°C/60s	72°C/10min	425	Michotey et al.and Throback et al.
	*nir*S-R	GASTTCGGRTGSGTCTTGA							
*nor*B	*nor*B-F	GGNCAYCARGGNTAYGA	57	95°C/5min; 95°C/30s, 57°C/45s, 72°C/60s	95°C/3min	94°C/30s, 57°C/45s, 72°C/60s	72°C/10min	262	Braker et al.
	*nor*B-R	ACCCANAGRTGNACNACCCACC A							
*nos*Z	*nos*Z-F	AGAACGACCAGCTGATCGACA	60	95°C/5min; 95°C/60s, 60°C/60s, 72°C/60s	95°C/3min	95°C/45s, 60°C/60s, 72°C/60s	72°C/10min	300	Henry et al.
	*nos*Z-R	TCCATGGTGACGCCGTGGTTG							
16S rRNA	16S-F	CCTACGGGAGGCAGCAG	55	95°C/5min; 95°C/30s, 55°C/45s, 72°C/50s	—	—	—	174	Lopez-Gutierrez et al.
	16S-R	ATTCCGCGGCTGGCA							

F: forward; R: revers

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