Supporting Information

for

Determination of growth kinetics of microorganisms linked with 1,4-dioxane degradation in a consortium based on two improved methods

Yi Xiong[a,¶], Boya Wang[a,¶], Chao Zhou[b], Huan Chen[c], Gang Chen[a], Youneng Tang[a,*]

a Department of Civil and Environmental Engineering, FAMU-FSU College of Engineering, Florida State University, Tallahassee, Florida 32310, United States

b Geosyntec Consultants Inc., Huntington Beach, California 92648, United States

c National High Magnetic Field Laboratory, Tallahassee, Florida 32310, United States

¶ The two authors contributed equally to this work.

* Corresponding author email: ytang@eng.famu.fsu.edu, phone: +1(850)410-6119

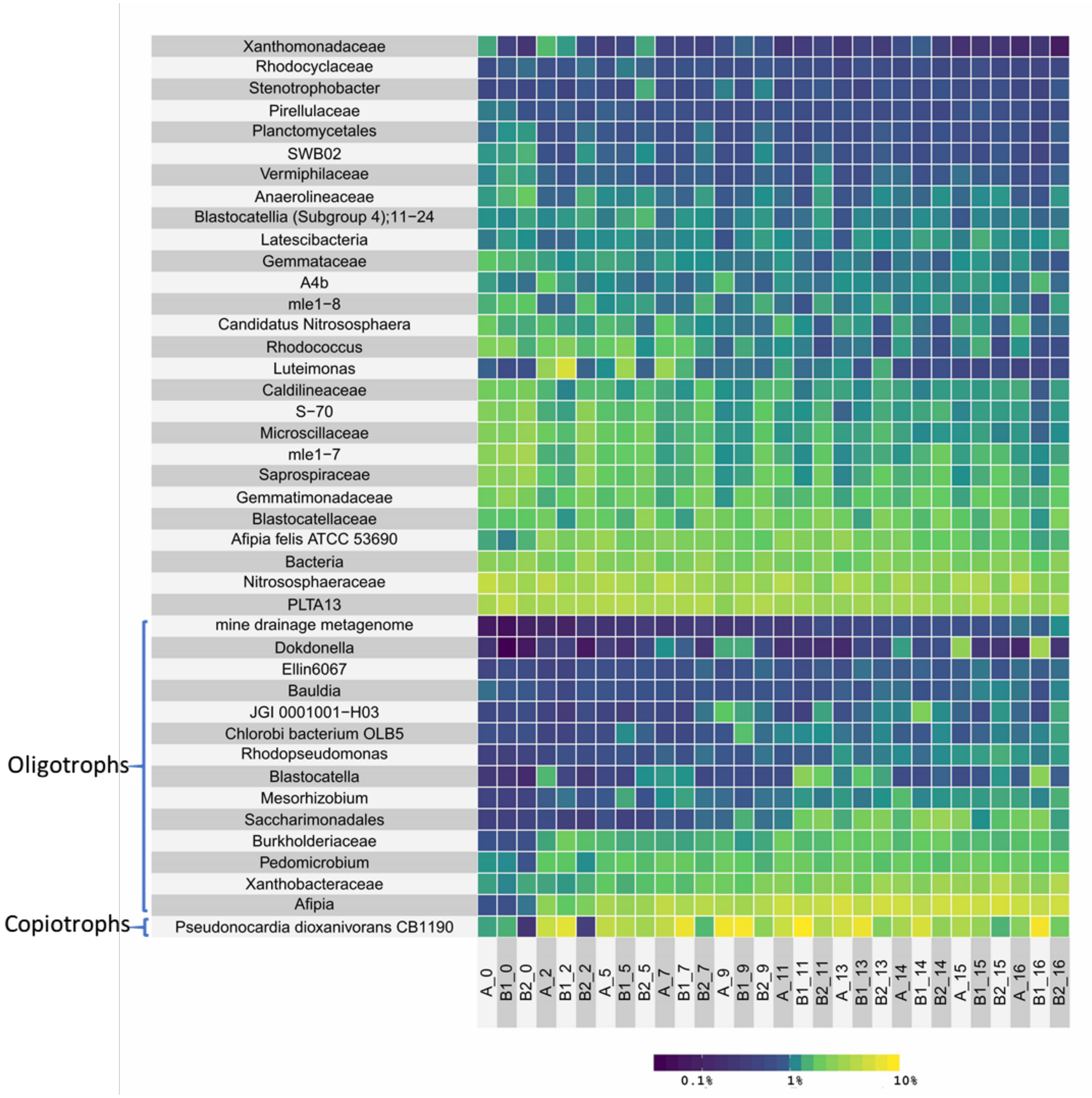


Figure S1. Heatmap for microbial groups with relative abundance >1% for at least one sample during the microcosm experiments for constants determination. (Notes: The sample corresponding to "A_0" was from Bottle A on Day zero, the sample corresponding to "B1_0" was the first sample from Bottle B on Day zero, and the sample corresponding to "B2_0" was the duplicate sample from Bottle B on Day zero.)

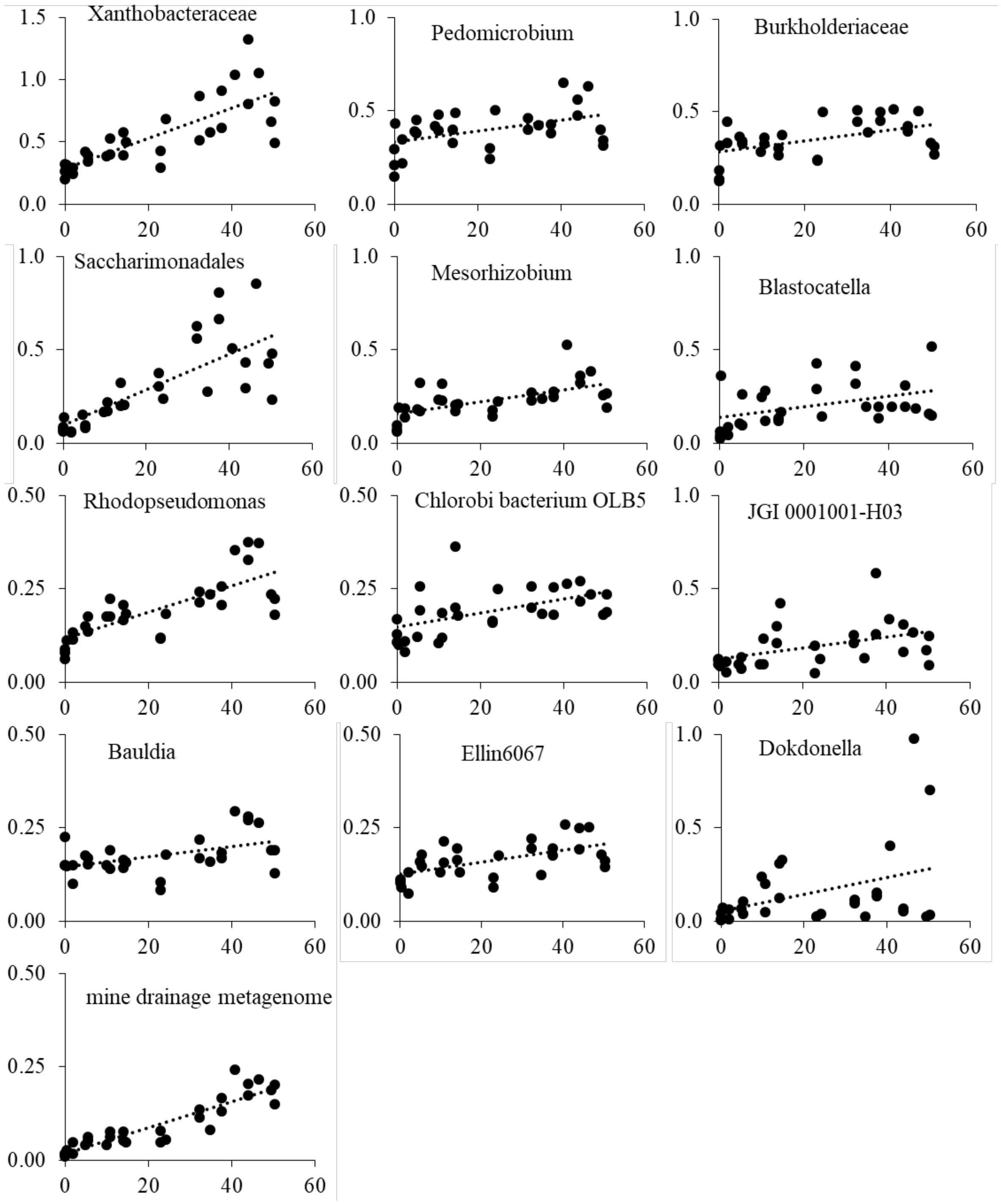
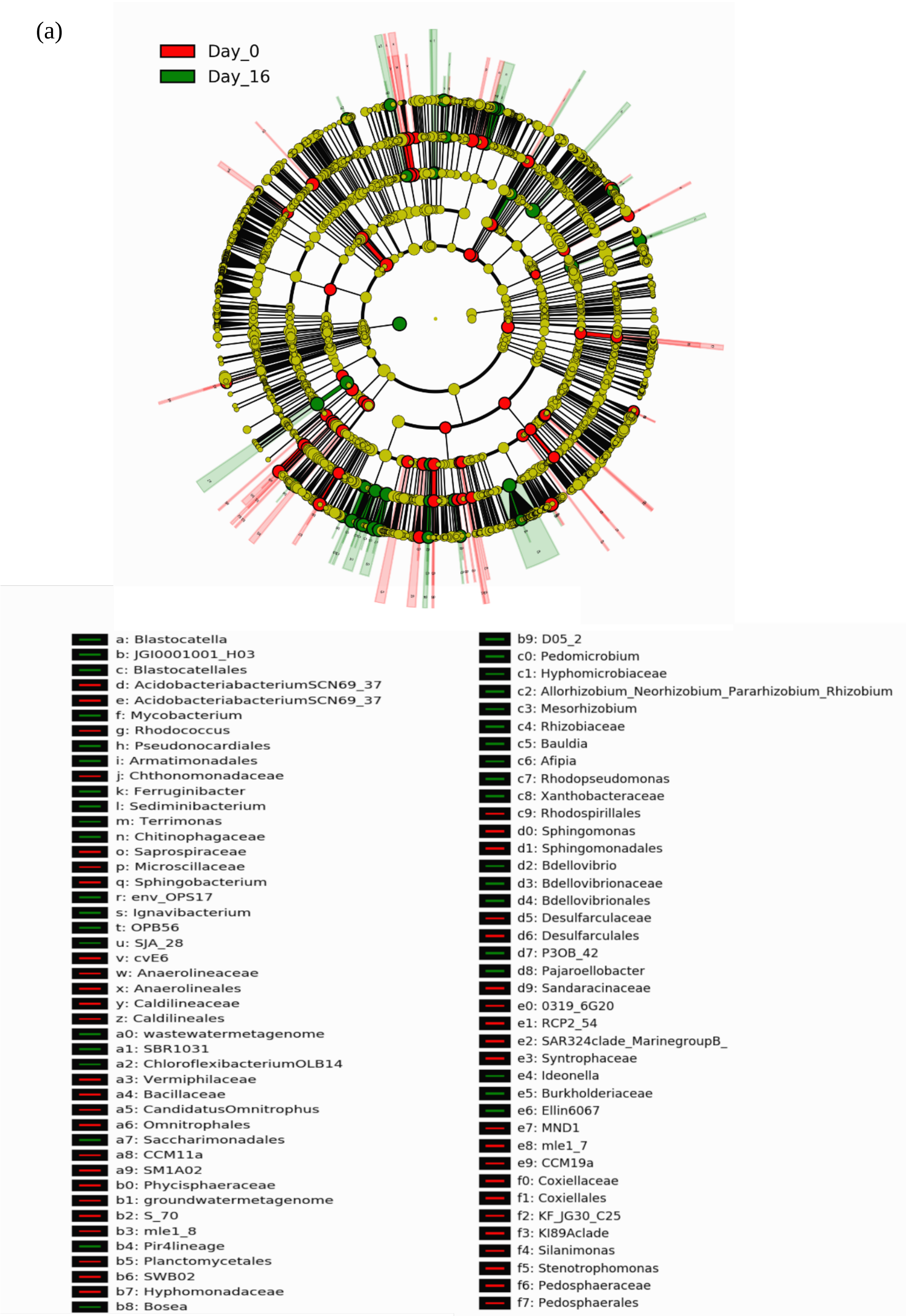


Figure S2. Linear regression analysis results for the other 13 microbial groups with p-value < 0.05 and coefficient > 0. (Notes: For each figure, the horizontal axis was the concentration of degraded dioxane (= dioxane concentration at Day 0 – dioxane concentration at a certain day, mg/L), and the vertical axis was the biomass concentration of the microbial group (mg protein/L). Note: The p-value and coefficient of each group are summarized in Table 2.)

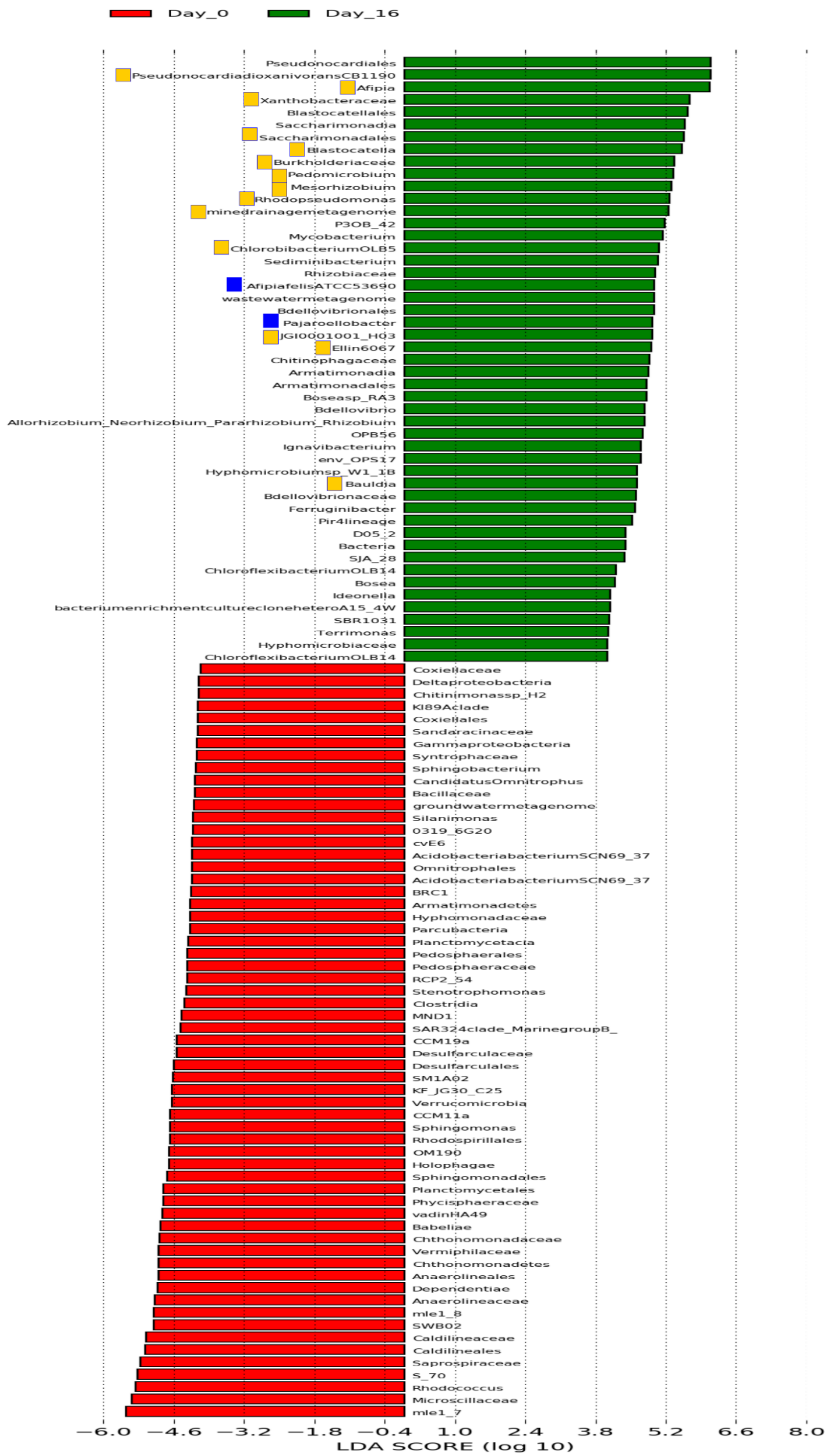
Table S1: Comparison of the three methods

	Conventional Method	Improved Method 1	Improve Method 2
Step 1. Microcosm Experiments for Constants Determination	√	√	√
Step 2. Microbial Community Analysis	×	√	√
Step 3. Analysis of Correlation between Dioxane and Various Groups of Bacteria	×	√	√
Step 4. Estimation of Constants in Kinetics	√ 1) Use total biomass concentration for calculation. 2) One set of kinetics parameters is estimated.	√ 1) Use the fraction of biomass linked with dioxane degradation (copiotrophs and oligotrophs are not separated) for calculation. 2) One set of kinetics parameters is estimated.	√ 1) Use the fraction of biomass linked with dioxane degradation (copiotrophs and oligotrophs are separated) for calculation. 2)Two sets of kinetics parameters are estimated: one set for oligotrophs and the other set for copiotrophs.
Step 5. Evaluation of the Estimated Constants in Kinetics	√	√	√

(a)


Day_0
Day_16
a: Blastocatella
b: JGI0001001_H03
c: Blastocatellales
d: AcidobacteriabacteriumSCN69_37
e: AcidobacteriabacteriumSCN69_37
f: Mycobacterium
g: Rhodococcus
h: Pseudonocardiales
i: Armatimonadales
j: Chthonomonadaceae
k: Ferruginibacter
l: Sediminibacterium
m: Terrimonas
n: Chitinophagaceae
o: Saprospiraceae
p: Microscillaceae
q: Sphingobacterium
r: env_OPS17
s: Ignavibacterium
t: OPB56
u: SJA_28
v: cvE6
w: Anaerolineaceae
x: Anaerolineales
y: Caldilineaceae
z: Caldilineales
a0: wastewatermetagenome
a1: SBR1031
a2: ChloroflexibacteriumOLB14
a3: Vermiphilaceae
a4: Bacillaceae
a5: CandidatusOmnitrophus
a6: Omnitrophales
a7: Saccharimonadales
a8: CCM11a
a9: SM1A02
b0: Phycisphaeraceae
b1: groundwatermetagenome
b2: S_70
b3: mle1_8
b4: Pir4lineage
b5: Planctomycetales
b6: SWB02
b7: Hyphomonadaceae
b8: Bosea
b9: D05_2
c0: Pedomicrobium
c1: Hyphomicrobiaceae
c2: Allorhizobium_Neorhizobium_Pararhizobium_Rhizobium
c3: Mesorhizobium
c4: Rhizobiaceae
c5: Bauldia
c6: Afipia
c7: Rhodopseudomonas
c8: Xanthobacteraceae
c9: Rhodospirillales
d0: Sphingomonas
d1: Sphingomonadales
d2: Bdellovibrio
d3: Bdellovibrionaceae
d4: Bdellovibrionales
d5: Desulfarculaceae
d6: Desulfarculales
d7: P3OB_42
d8: Pajaroellobacter
d9: Sandaracinaceae
e0: 0319_6G20
e1: RCP2_54
e2: SAR324clade_MarinegroupB_
e3: Syntrophaceae
e4: Ideonella
e5: Burkholderiaceae
e6: Ellin6067
e7: MND1
e8: mle1_7
e9: CCM19a
f0: Coxiellaceae
f1: Coxiellales
f2: KF_JG30_C25
f3: KI89Aclade
f4: Silanimonas
f5: Stenotrophomonas
f6: Pedosphaeraceae
f7: Pedosphaerales

(b)



Day_0
Day_16
Pseudonocardiales
PseudonocardiadioxanivoransCB1190
Afipia
Xanthobacteraceae
Blastocatellales
Saccharimonadia
Saccharimonadales
Blastocatella
Burkholderiaceae
Pedomicrobium
Mesorhizobium
Rhodopseudomonas
minedrainagemetagenome
P3OB_42
Mycobacterium
ChlorobibacteriumOLB5
Sediminibacterium
Rhizobiaceae
AfipiafelisATCC53690
wastewatermetagenome
Bdellovibrionales
Pajaroellobacter
JGI0001001_H03
Ellin6067
Chitinophagaceae
Armatimonadia
Armatimonadales
Boseasp_RA3
Bdellovibrio
Allorhizobium_Neorhizobium_Pararhizobium_Rhizobium
OPB56
Ignavibacterium
env_OPS17
Hyphomicrobiumsp_W1_1B
Bauldia
Bdellovibrionaceae
Ferruginibacter
Pir4lineage
D05_2
Bacteria
SJA_28
ChloroflexibacteriumOLB14
Bosea
Ideonella
bacteriumenrichmentculturecloneheteroA15_4W
SBR1031
Terrimonas
Hyphomicrobiaceae
ChloroflexibacteriumOLB14
Coxiellaceae
Deltaproteobacteria
Chitinimonassp_H2
KI89Aclade
Coxiellales
Sandaracinaceae
Gammaproteobacteria
Syntrophaceae
Sphingobacterium
CandidatusOmnitrophus
Bacillaceae
groundwatermetagenome
Silanimonas
0319_6G20
cvE6
AcidobacteriabacteriumSCN69_37
Omnitrophales
AcidobacteriabacteriumSCN69_37
BRC1
Armatimonadetes
Hyphomonadaceae
Parcubacteria
Planctomycetacia
Pedosphaerales
Pedosphaeraceae
RCP2_54
Stenotrophomonas
Clostridia
MND1
SAR324clade_MarinegroupB_
CCM19a
Desulfarculaceae
Desulfarculales
SM1A02
KF_JG30_C25
Verrucomicrobia
CCM11a
Sphingomonas
Rhodospirillales
OM190
Holophagae
Sphingomonadales
Planctomycetales
Phycisphaeraceae
vadinHA49
Babeliae
Chthonomonadaceae
Vermiphilaceae
Chthonomonadetes
Anaerolineales
Dependentiae
Anaerolineaceae
mle1_8
SWB02
Caldilineaceae
Caldilineales
Saprospiraceae
S_70
Rhodococcus
Microscillaceae
mle1_7
−6.0
−4.6
−3.2
−1.8
−0.4
1.0
2.4
3.8
5.2
6.6
8.0
LDA SCORE (log 10)

Figure S3. (a) Taxonomic cladogram obtained using LEfSe (Linear discriminant analysis Effect Size, Segata *et al.*, (2011)) analysis of the 16S rRNA sequences. It shows taxa that meet a significant LDA (Linear Discriminant Analysis) threshold value of >4, and indicates the phylogenetic distribution of microbial lineages associated with 1,4-dioxane degradation. The highlighted taxa indicate enrichment of the taxa on the day that corresponded to the highlighting color (green versus red shading). Circles represent phylogenetic levels from phylum (center) to genus (rim). Each circle's diameter is proportional to the taxon's abundance. (b) Histogram of taxa with LDA > 4. The following notes apply to Day 16. The 14 orange squares represent 14 taxa that were identified through the LEfSe analysis and the correlation analysis in Section 3.3 (*i.e.*, correlation between 1,4-dioxane degradation and OTU enrichment). The only one taxon (of the total 15 taxa identified by the correlation analysis) that was not identified by the LEfSe analysis was *Dokdonella*. The two blue squares represent two taxa identified through the LEfSe analysis, but not identified through the correlation analysis. The 17 taxa (= 14 + 1 + 2) discussed so far had an abundance of > 1% each. The abundance of the other 33 taxa was less than 1% each. They were identified through the LEfSe analysis, but not considered in the correlation analysis in Section 3.3. The largely overlapping between the correlation analysis results (14/15 = 93%) and the LEfSe analysis results with high LDA scores (see orange squares) suggests that we have captured most of the abundant taxa (>1%) linked with 1,4-dioxane degradation by using the correlation analysis method. The inconsistence between the correlation analysis results and the LEfSe analysis results suggests that we may have neglected many taxa that played a role in 1,4-dioxane degradation but had low abundance (<1%).

References

Segata N, Izard J, Walron L, Gevers D, Miropolsky L, Garrett W, Huttenhower C (2011). Metagenomic biomarker discovery and explanation. Genome Biology, 12 (6): R60.