Cross-feeding between *Thauera aminoaromatica* and *Rhodococcus pyridinivorans* drove quinoline biodegradation in a denitrifying bioreactor

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Running title: Syntrophic denitrifying degradation of quinoline

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Abstract

The complex bacterial community is predominated by several taxa, such as *Thauera* and *Rhodococcus*, in a quinoline-degrading denitrifying bioreactor. Yet it remains unclear about how the interactions between the different bacteria mediate the quinoline metabolism in denitrifying condition. In this study, we designed a sequence-specific amplification to guide the isolation of the most predominant bacteria and obtained four strains of *Thauera aminoaromatica*, the representative of one key member in the bioreactor. Test on these isolates demonstrated that all of them were unable to strive on quinoline but could efficiently degrade 2-hydroxyquinoline, the hypothesized primary intermediate of quinoline catabolism, under nitrate-reducing condition. However, another isolate, *Rhodococcus pyridinivorans* YF3, corresponding to the second abundant taxon in the same bioreactor, was found to degrade quinoline via 2-hydroxyquinoline. The end products and removal rate of quinoline by isolate YF3 were largely varied with the quantity of available oxygen. Specifically, quinoline could only be converted into 2-hydroxyquinoline without further transformation under the condition with insufficient oxygen, e.g. less than 0.5% initial oxygen in the vials. However, if were aerobically pre-cultured in the medium with quinoline the resting cells of YF3 could anaerobically convert quinoline into 2-hydroxyquinoline. A two-strain consortium constructed with isolates from *Thauera* (R2) and *Rhodococcus* (YF3) demonstrated an efficient denitrifying degradation of quinoline. Thus, we experimentally proved that the metabolism interaction based on the 2-hydroxyquinoline cross-feeding between two predominant bacteria constituted the mainstream of quinoline degradation. This work sheds light on the understanding of mechanism of quinoline removal in the denitrifying bioreactor.

Keywords：Quinoline-degrading bioreactor, 2-hydroxyquinoline, microbial interaction, cross-feeding, oxygen, denitrification

Importance

We experimentally verified the most predominant *Thauera* sp. was indeed active degrader for the intermediate metabolites and the second abundant taxon *Rhodococcus* exerted, however, key function for opening the food box for a complex quinoline-degrading community. An ecological guild composed of two isolates was assembled, revealing the different roles of keystone organisms in the microbial community. This study, to our best knowledge, is the first report on the cross feeding between the initial attacker with unprofitable catalysis of reluctant heterocyclic compounds and the second bacterium which then completely degrade the compound transformed by the first bacterium. These results could be a significant step forward towards elucidation of microbial mechanism for quinoline denitrifying degradation.

Introduction

Quinoline and its derivatives are typical N-heterocyclic compounds that occur widely in coal tar, shale oil and creosote, and serve as raw materials and solvents in chemical, pharmaceutical and pesticide industries (1). They are known to be carcinogenic and mutagenic to human (2,3) and aroused a significant concern as recalcitrant pollutants to ecological environment.

Anaerobic bioremediation is an attractive technology due to its virtue of energy saving and cost-effectiveness, since heavily contaminated environments are often oxygen deficient (4). However, most of the literatures focused the aerobic degradation of quinoline. Various microorganisms capable of metabolizing quinoline aerobically have been isolated, mostly belonging to *Pseudomonas* (5-7), *Rhodococcus* (8) and *Bacillus* (9). The pathways of aerobic quinoline degradation have also been well described (10). However, little attention was paid to the anaerobic quinoline biodegradation. Degradation of quinoline in industrial scale wastewater treatment reactors had been reported in a few literature (11,12). In addition, no evidence proved the role of main anaerobic degraders in these industrial bioreactors. There were several efforts to elucidate the anaerobic degraders by using the lab scale bioreactor

and batch culture experiments (13-16). But to date, only one isolate, *Desulfobacterium indolicum* strain DSM 3383, which used sulfate as electron acceptor, was purely cultured as an anaerobic quinoline degrader (17). It is wrapped in mystery why there is few isolate for anaerobic quinoline degradation.

An efficient anoxic microbial community was enriched in a chemostat that was operated for more than 10 years with quinoline as electron donor and nitrate as electron acceptor. Phylogenetic analysis of this consortium showed that specific phylotypes were associated with different stages of the degradation (16), which suggests that microorganisms interact during quinoline metabolism. However, our understanding of this interaction is restricted due to the complexity of the community composition in the bioreactor while lack of anaerobic degrading microorganisms available in pure culture. Therefore, functional analysis using the representative isolates would be a crucial further step for understanding the quinoline denitrifying degradation in the reactor.

To investigate the underlying microbial processes in this complex quinoline-degrading consortium, we endeavored to isolate the most predominant and active bacteria, which had been identified as the keystone organisms involved in denitrifying quinoline removal (16,18). The degradation characteristics of these isolates were evaluated under different conditions. Based on the degradation function of different isolates, a co-culture of representative isolates of *Thauera* and *Rhodococcus* were constructed to demonstrate the cooperation of two bacteria during quinoline metabolism under defined conditions.

Materials and methods

Operation of the quinoline denitrifying bioreactor

A 2-L tank, filled with plastic rings and synthetic fiber strings as semisoft media, was used to construct a lab-scale bioreactor. Seeding sludge was collected from an anoxic tank of a coking wastewater treatment plant from the Shanghai Coking and Chemical Factory (Wujing, Shanghai). The synthetic wastewater was composed of

quinoline (100 mg/L), $NaNO_3$ (240 mg/L) and K_2HPO_4 (140 mg/L). During wastewater upflowing (i.e. from the bottom to up) without any agitation, the inner of reactor was oxygen-depleted due to the rapid consumption by aerobic bacteria. The hydraulic retention time was 24 h. The pH and temperature of the reactor were adjusted and controlled at 7.5 and 25 °C, respectively.

Profiling of the quinoline-degrading bacterial community

The biofilm sample was collected by scraping the biofilm from the surface of the supporting materials in the bioreactor. Genomic DNA extraction of the samples in triplicate was conducted as previously described (19). The sequencing library of V3-V4 regions in the 16S rRNA gene were constructed by two-step PCR amplification according to Illumina's instructions. The purified amplicons were sequenced using the Illumina MiSeq System (Illumina Inc., United States). The preliminary process of the raw sequencing data was conducted as the previous document (20). Sequence assembly was first implemented, and the unique sequences obtained by dereplication were sorted by decreasing abundance, and then singletons were abandoned. UPARSE's default (21) was used to select the representative operational taxonomy units (OTUs), and UCHIME (22) was selected to further perform reference-based chimera detection against the RDP classifier training database (23). Finally, the OTU table was completed by mapping quality-filtered reads to the representative OTUs with Usearch (24), resulting in a global alignment algorithm at a 97% cutoff. Further analysis was performed using the QIIME platform (version 1.8)(25). In addition, representative sequences for each OTU were submitted to the online RDP classifier (RDP database version 2.11) to determine the phylogeny, with a bootstrap cutoff of 80%. The 16S rRNA gene sequences in this study were submitted to the GenBank Sequence Read Archive (SRA) database in the National Center for Biotechnology Information (NCBI) under the accession number SRP188486.

Isolation and identification of the most predominant bacteria

Six types of media were used to isolate the most predominant *Thauera* spp. strains. The composition of the six media were described as follows, Nutrient Agar

(NA): peptone 10 g/L, beef extract 3 g/L, NaCl 5 g/L, agar 15 g/L (26); 1/10 strength NA: diluted by 10 folds based on NA except for the content of agar; Tryptic Soy Agar (TSA): tryptone 17 g/L, soy peptone 3 g/L, glucose 2.5 g/L, NaCl 5 g/L, K_2HPO_4 2.5 g/L, agar 15 g/L (27); 1/10 strength TSA: diluted by 10 folds based on TSA except for the content of agar; Reasoner's 2A agar (R2A): yeast extract 0.5 g/L, peptone 0.5 g/L, casein hydrolysate 0.5 g/L, glucose 0.5 g/L, soluble starch 0.5 g/L, KH_2PO_4 0.3 g/L, $MgSO_4$ 0.024 g/L, sodium pyruvate 0.3 g/L, agar 15 g/L (27); Waste Water Medium (WWM): made by raw wastewater which was filtrated and removed bacteria and supplemented with 1.5% agar.

The specific PCR primer targeted the most predominant *Thauera* sp. was designed by DNAMAN (version 7.0) using the sequences of different OTUs belonging to genus *Thauera,* which obtained from a full length 16S rRNA clone library constructed for quinoline denitrifying bioreactor (18). OTU specific primers were used to assist the screening of the target *Thauera* strains following the previously described procedure (13).

The biofilm sample was obtained and mixed in a shaker with 150 rpm for 2 h. Then the suspension was diluted and spread on the plate of six types of media including NA, 1/10 strength NA, TSA, 1/10 strength TSA, R_2A and WWM. All plates were incubated at 30 °C under aerobic and anaerobic conditions, respectively. The isolates that had positive signal of colony PCR were purified by plate-streaking technology. Finally, genomic DNA of all isolates were extracted by phenol-chloroform protocol (28). The ERIC-PCR was conducted to have genomic typing of all isolates (29). And representative strains for different type of ERIC fingerprints were selected for 16S rRNA gene sequencing to identify the taxonomy and construct phylogenetic tree. The 16S rRNA gene sequences of representative strains were deposited in GenBank with the accession numbers of MK271352 (R2), MK272943 (R4), MK272944 (N15), MK272945 (N38), respectively.

Pre-culture of bacterial inoculum for biodegradation experiment

Unless specific mention, the inoculum for all experiments was prepared by inoculating the strains of *Thauera* spp. or *Rhodococcus pyridinivorans* YF3

(GU143680.1) in the Nutrient Broth medium (NB) and incubating at 30 °C, and 150 rpm on a rotary shaker for 24 h. The bacterial cells were harvested by centrifugation and washed three times in mineral salt medium (MSM) and the suspension was used as inoculum. The mineral salt medium (MSM) contained: $K_2HPO_4 \cdot 3H_2O$ 0.57 g/L, KH_2PO_4 0.233 g/L, NH_4Cl 0.02675 g/L, NaCl 0.5 g/L, $NaNO_3$ 85 mg/L (1mM) and trace elements including $NaHCO_3$ 0.168 g/L, $MgSO_4$ 0.12 g/L, $CaCl_2$ 0.0544 g/L, disodium EDTA 0.025 g/L, H_3BO_3 0.0036 g/L, $FeSO_4 \cdot 7H_2O$ 0.0015 g/L, $CoCl_2$ 0.0012 g/L, $Ni(NH_4)_2(SO_4)_2$ 0.0012 g/L, Na_2MoO_4 0.00094 g/L, Na_2SeO_4 0.00026 g/L, $MnSO_4$ 0.0002 g/L, $ZnSO_4$ 0.00016 g/L, $CuSO_4$ 0.000032 g/L, pH 7.4.

Degradation experiments for bacterial isolates

The batch experiments were conducted using a series of 20 mL headspace vials, which contained 10 mL of either the MMQ or MMHQ medium. The composition of the MMQ or MMHQ were described as follows. MMQ: mineral salt medium (MSM) supplemented with 0.23 mM Quinoline (Sigma–Aldrich, Co., Inc); MMHQ: mineral salt medium (MSM) supplemented with 0.23 mM 2-hydroxyquinoline (Sigma-Aldrich, Co., Inc).

The inoculum was 5% to 10% of volumetric proportion. For each of the different media, vials without inoculum but maintained under the same condition were set as the abiotic negative control. All vials were immediately sealed with airtight butyl-rubber septa and aluminum crimp caps and made anoxic by repeated evacuation and filling with helium. To investigate the effects of pH value on the degradation, the initial pH of media was adjusted to 4.5, 6.5, 7.5, 8.5 and 10.5, respectively.

To investigate the effects of oxygen on quinoline biotransformation by *Rhodococcus* sp. YF3, 0.04 mL, 0.10 mL and 0.16 mL pure oxygen were respectively injected into the vials by precision syringes, named as the groups of 0.2% O_2, 0.5% O_2, 0.8% O_2, respectively. The headspace of anaerobic vials was no oxygen injection while the headspace of aerobic vials was replaced by air. All vials were incubated in the dark at 30 °C with shaking at 150 rpm. Samples were taken periodically by syringes in anaerobic workstation to prevent from oxygen during incubation. All treatments in the above degradation experiment had triplicates to minimize the

194 experimental variation.

195 **Measurement of chemical compounds**

196 The sampled liquid was centrifuged and the supernatant was used for further
197 analysis. The concentration of quinoline and 2-hydroxyquinoline were analyzed by
198 HPLC system (Agilent, ZORBAX SB-C18 reverse-phase column, 150 × 4.6 mm,
199 5μm). The mobile phase was methanol solution with a volume ratio of 60:40
200 (methanol / water) at a flow rate of 1.0 mL/min. Quinoline and 2-hydroxyquinoline
201 were both detected at 225 nm wavelength. The injection volume was 20 μL and
202 column temperature was at 30 °C. The nitrate concentration was measured using a
203 PXJ-1B ion meter (Jiangfen, China) with a pNO_3^{-1} nitrate ion-selective electrode
204 (Tianci, Shanghai).

205

206 # Results

207 **Profiling of the quinoline-degrading bacterial community**

208 The removal rates of quinoline and nitrate maintained stably at about 80% and
209 90%, respectively, in the denitrifying quinoline-degrading bioreactor. Before sample
210 collection for isolating the most predominant organisms, the bacterial community of
211 triplicate samples from the reactor was profiled by high-throughput sequencing. The
212 Illumina Miseq platform yielded 73902 high-quality reads with an average of 24634
213 reads per sample (±1418 SD). 460 species-level operational taxonomic units (OTUs)
214 were delineated using 97% identity as cut-off value. Six predominant OTUs
215 contributed to 52.06% of all reads (Fig. 1). The most predominant OTU (OTU1) with
216 relative abundance of 22.5% belonged to genus *Thauera*; while OTU2, the second
217 abundant OTU (12.5%), was affiliated to genus *Rhodococcus.*

218 **Isolation and identification of the most predominant bacteria**

219 Since that the most predominant OTU was supposed as the key player for the
220 quinoline degradation, we attempted to isolate the corresponding bacteria from the
221 bioreactor using sequence-guiding isolation strategy. The specific PCR primers based
222 on most predominant *Thauera* sp. were designed in silico. After selection based on the

amplification, the primer pair of Thau135/Thau716 (5'-GGGATAACGTAGCGAAAGCT-3'; 5'-CCATCGGTGTTCCTCCTG-3') was further evaluated experimentally for its specificity. The amplicon of reactor sample by using the selected primer pair was cloned and 15 clones were randomly selected for sequencing of partial 16S rRNA gene with length longer than 500 bp. Among them, 8 clones showed 100% identity with DR-80 and most predominant OTU1, and the other 5 clones shared over 99.7% identity with DR-80/OTU1, only 2 clones showed identity about 99.5%. These results indicated that the specificity of Thau135/Thau716 was high enough to guide the following isolation.

Several hundreds of colonies grown on the plates of six different media were screened by specific amplification using the primers of Thau135/Thau716. Totally, 13 positive colonies were picked, of which 3 were purified from R2A medium, 6 from 1/10 strength TSA and 4 from 1/10 strength NA medium. Morphologically, these selected colonies were pearl-like on the 1/10 NA plate and the cells formed characteristic flocs or clusters in NB liquid culture shaking with 150 rpm at 30 ºC. All of these isolates were classified into four genotypes by ERIC-PCR result, in which the isolates of R2, R4, N15 and N38 were selected as the representative strains for each ERIC type. The full length of 16S rRNA gene sequences of four representative strains were 100% identity with each other and had one nucleotide mismatch with the uncultured *Thauera* clone DR-80, which had highest abundance in a lab scale bioreactor for quinoline degradation (18). Besides, all isolates showed 100% identity with the 16S rRNA gene sequence of the most predominant *Thauera* genome which was assembled using the metagenome data for the same bioreactor in our previous work (30). Moreover, *Thauera aminoaromatica* strain S2 was the closest neighbor of all isolates when blast 16S rRNA gene sequences with NCBI nr database (Fig. 2), which was consistent with the previous report about the metagenome data that the assembled genome of the most predominant organism in the reactor was most closely related to *Thauera aminoaromatica* S2 (30). These four isolates phylogenetically discriminated with other three isolates, 3-35, Q4, Q20-C, which were obtained previously from a quinoline containing coking wastewater denitrifying treatment

bioreactor from which the seeding sludge for our bioreactor was obtained (14).

Metabolism of Quinoline and 2-hydroxyquinoline by *Thauera* isolates

Firstly, the isolated *Thauera* strains were tested for their capacity of quinoline degradation. Unexpectedly, none of them could metabolize quinoline, when it was used as sole carbon source, both under anaerobic and aerobic conditions. However, all four isolates could utilize 2-hydroxyquinoline, the derivative of quinoline, which was reported as the first degrading intermediate of quinoline (31). Data showed that about 80% of 50 mg/L 2-hydroxyquinoline was eliminated in MMHQ medium within 10 days under nitrate-reducing condition and no any accumulation of intermediates in the measured samples (Fig. 3a). The effects of pH value on 2-hydroxyquinoline degradation were further investigated. The results showed that 30 mg/L 2-hydroxyquinoline was depleted in 132 h both under pH 7.5 and 8.5 conditions (Fig. 3b). Degradation of substrates was apparently inhibited under acidic (pH≤6.5) or alkaline (pH≥10.5) condition. The transformation of 2-hydroxyquinoline was pH-dependent, with the optimal pH range from 7.5 to 8.5. Additionally, although all isolates had capacity for 2-hydroxyquinoline removal, their rates for degradation were different. For example, the isolate N38 had the lowest rate, whereas the isolate R2 had the highest rate of 0.583 mg/L.h.

Metabolism of quinoline by a *Rhodococcus* isolate

On the petri dish for bacteria isolation, a type of reddish colony frequently appeared. The identification by 16S rRNA gene suggested it represented another predominant bacterial phylotype for quinoline degradation proposed in previous literature (16). The isolate YF3 was selected as the representative of the most predominant *Rhodococcus* OTU, which was identified as *Rhodococcus pyridinivorans*. Transformation of quinoline by YF3 under different amount of initial oxygen in headspace of vials was explored. We found that the quinoline removal rate and transformed products were varied due to the different condition of oxygen supplement. After 24 h incubation, the degradation of microcosms which inoculated with the inoculum grown on NB medium was analyzed. The results showed that 0.23 mM quinoline was completely oxidized when supplemented with sufficient oxygen; in

283 contrast, quinoline was persistent under anaerobic condition; whereas, about 0.09 mM
284 2-hydroxyquinoline accumulated and 0.13 mM quinoline remained if 0.2%
285 oxygen supplied, which equal to 2.8 mg/L dissolved oxygen; quinoline was
286 completely transformed into 2-hydroxyquinoline under 0.5% oxygen,, which equal to
287 7.1 mg/L dissolved oxygen; when the oxygen concentration increased to 0.8%, which
288 equal to 11.2 mg/L dissolved oxygen, only 0.17 mM 2-hydroxyquinoline retained due
289 to the further degradation (Fig. 4).

290 **Synergistic degradation of quinoline by a bacterial co-culture**

291 Since that *Thauera* sp. R2 has been proved as an anaerobic 2-hydroxyquinoline
292 degrader and *Rhodococcus* sp. YF3 had the capacity for hydroxylation of quinoline,
293 we assumed that the degradation of quinoline might be accomplished in the bioreactor
294 via metabolic cooperation of these two bacteria. Thus, we performed a trial of
295 co-cultivation using the above mentioned bacterial strains of *Thauera* and
296 *Rhodococcus* to test the capacity of degradation of quinoline under the conditions of
297 different oxygen-availability. We found that 0.07 mM and 0.01 mM quinoline
298 remained in the vials of experimental groups of 0.2% O_2 and 0.5% O_2, respectively.
299 2-hydroxyquinoline was finally eliminated in all the groups within 48 h while
300 quinoline was completely depleted only in 0.8% O_2 (Fig. 5a).

301 Considering the original bioreactor is still an oxygen deficient system, we try to
302 elucidate the possible mechanism of anaerobic quinoline degradation. We have
303 verified that *Rhodococcus* sp. YF3 could hydroxylate quinoline under micro-oxygen
304 condition. Washing and transferring YF3 cells, which were pre-incubated with
305 quinoline, 2-hydroxyquinoline or phenol for 24 h under 0.5% oxygen condition, to
306 fresh MMQ medium and incubating for another 72 h under completely anaerobic
307 condition, we observed that 0.23 mM quinoline was basically transformed into
308 equivalent 2-hydroxyquinoline by YF3 monoculture. 2-Hydroxyquinoline was further
309 removed in the co-culture of YF3 and R2 (Fig. 5b).

310

311 ## Discussion

In this study, we aimed to elucidate the microbial mechanism of quinoline denitrifying degradation. As a first step, we tried to isolate the most predominant bacteria in the quinoline-degrading consortium, which was considered as the promising anaerobic quinoline degrader. However, many attempts failed at isolating the target strain by using the media with quinoline as sole carbon source. In the earlier study, a *Thauera* genus-specific nested-PCR denaturing gradient gel electrophoresis (DGGE) method was developed, combining with media containing various carbon source, to guide the isolation of *Thauera* spp.. In that study, three *Thauera* strains (Q4, Q20-C, and 3-35) were obtained but none of them could utilize quinoline either aerobically or anaerobically (13,14). Considering that the former isolates were corresponding to the less abundant *Thauera* species in the bioreactor, in this study, we designed OTU specific PCR primers targeting the most predominant *Thauera* OTU1, as the biomarker to guide the isolation of the target *Thauera* bacterial strains. The colonies grown on various media were screened by using this method. As a result, four isolates belonged to *Thauera aminoaromatica*, were identified as the homologue of the most predominant bacteria in quinoline degrading bioreactor. *Thauera aminoaromatica* had been reported as one of denitrifying species capable of growing with amino-aromatic compounds (32). In this study, all isolates belonging to *Thauera aminoaromatica* were able to degrade 2-hydroxyquinoline but not quinoline under denitrifying conditions, which exactly explained previous unsuccessful attempts via the strategy of quinoline-degrading function-guiding isolation by using quinoline as the solo carbon source. Hence, sequence-guiding approach to assist isolating some difficult-to-culture bacteria could be an alternative and promising method.

Dozens literatures have reported that isolated bacteria may lack of the capability of complete mineralization of hydrocarbon, especially for the recalcitrant compounds (33). Moreover, regardless of present or absent of oxygen, the first metabolite of quinoline was 2-hydroxyquinoline (10). This compound was thus preferentially considered as substrate of most predominant *Thauera* bacteria in the denitrifying bioreactor. The results of 2-hydroxyquinoline degradation by monoculture of *Thauera* isolates in this study proved our hypothesis.

342 To further identify the bacteria being responsible for transforming quinoline to
343 2-hydroxyquinoline, we further explored other key players from the predominant
344 bacteria in the community. The members of genus *Rhodococcus* were known as
345 aerobic quinoline degraders (8), and this phylotype has been reported closely related
346 with quinoline denitrifying degradation (15, 16). Based on that, *Rhodococcus*
347 *pyridinivorans* YF3 isolated from the bioreactor was considered as a candidate to
348 initial attack for quinoline in the denitrifying bioreactor. Unsurprisingly, the
349 experimental result was well in accord with our speculation. Notably, the
350 consumption of quinoline by strain YF3 depended upon the oxygen concentrations.
351 Therefore, long-term accessing to low oxygen likely shape a *Rhodococcus*-dominated
352 community in the quinoline denitrifying bioreactor (15).

353 Communities of bacteria are extraordinarily complex with hundreds of interacting
354 taxa but the knowledge about how the tangled interactions within natural bacterial
355 communities mediate ecosystem functioning is limited (34). A promising way to
356 surpass this limitation is to create a synthetic community by artificially co-culturing of
357 selected two or more species under a well-defined media (35). Considering that the
358 metabolite of quinoline transformed by isolate YF3 could be further metabolized by
359 isolate R2, we constructed a synthetic consortium using pure cultures of these two
360 isolates to achieve cooperative degradation of quinoline. In this consortium, *Thauera*
361 sp. R2 cross-feed on the 2-hydroxyquinoline released by *Rhodococcus* sp. YF3.
362 Ecologically, this type of microbial interaction is equivalent to a commensalism (36).
363 Here the initial step of quinoline degradation is hydroxylation at the 2 position which
364 requires energy (37), but not provides any substrate and energy for growth.
365 Accordingly, *Rhodococcus* sp. YF3 would not benefit from the hydroxylation reaction.
366 In this study, the product 2-hydroxyquinoline of the first transformation step served as
367 the substrate for the next organism in the chain. Meanwhile, *Thauera* sp. R2 released
368 some intermediates during further metabolism. And the bacteria of *Rhodococcus* sp.
369 YF3 could utilize these metabolites leaked from *Thauera* cells to obtain energy and
370 for growth. Consequently, most of the carbon from quinoline was consumed by R2
371 cells and small portion by YF3 and other bacteria. It was well consistent with the

372 highest abundance of *Thauera* sp. R2 and relatively lower abundance of *Rhodococcus*
373 sp. YF3 in the bioreactor, although the latter acted like a lord to open the food box for
374 *Thauera* R2. Thus, the cross-feeding and interdependence between these two strains
375 was main driven force for quinoline denitrifying degradation and assembly for a
376 stable community in the denitrifying bioreactor. We summarized the ecological
377 mechanism of quinoline denitrifying degradation in Figure 6.

378 Moreover, it was worth considering why this multi-strain degradation occurred in
379 the quinoline-degrading reactor. In previous research (38), main reactions with respect
380 to starch biodegradation occurred at the lower part of reactor while the small
381 molecules such as acetate were converted to methane at the upper part. The organic
382 compounds were gradually attenuated along the upflow direction. It was speculated
383 that in our closed reactor, during the synthetic wastewater upflowing (i.e. from the
384 bottom to up), the dissolved oxygen coming with the synthetic wastewater (ca. 7 mg/L
385 dissolved oxygen) was rapidly consumed by *Rhodococcus* and other aerobic bacteria
386 in the bottom of reactor, which then made an anoxic denitrifying niche in the inner
387 space of bioreactor for *Thauera* to degrade 2-hydroxyquinoline that was produced by
388 hydroxylation via *Rhodococcus* activity under limited oxygen or anoxic condition.

389 Metabolic cooperation between/among different bacterial members in the
390 community are beneficial to the competitiveness. For instance, it has been shown that
391 degradation of 4-chlorodibenzofuran by *Sphingomonas* sp. RW1 results in the
392 accumulation of a dead-end product 3,5-dichlorosalicylate, while the inoculation with
393 *Burkholderia* sp. JWS enables the cooperative complete degradation (39). A
394 consortium comprised of *Escherichia coli* SD2 and *Pseudomonas putida* KT2440
395 pSB337 efficiently degrades parathion without accumulation of toxic intermediates
396 (40). Besides, it has also been reported that four members were actively involved in
397 the degradation of 4-chlorosalicylate, in which the strain of MT4 alleviated the stress
398 of MT1 by transforming the toxic intermediate protoanemonin (41). These examples
399 indicated that the metabolic complementarity in the microbial community were in
400 favor of efficient bioremediation to contaminants and also benefited for the better
401 adaptation or survival of bacteria in the specific environment.

This study collectively discussed commensal interaction in a quinoline-degrading consortium, aiming at highlighting importance of cross-feeding relationship between/among bacteria in biodegradation of organic compounds in the environment. Although our knowledge of this commensal relationship is not yet complete, understanding of such complex microbial interaction would provide useful information for assessing the biodegradability of organic compounds in the natural environment, for instance, the quinoline denitrifying degradation. Here we provided an unexpected and amazing insight into the microbial interaction. *Thauera aminoaromatica* R2, which adapted to denitrifying niche, could cooperate with *Rhodococc*us *pyridinivorans* YF3 to form a cross-feeding guild. To our best knowledge, this is the first report on cooperative relationship in quinoline denitrifying degrading guild, where two cross-feeding bacterial strains cooperated to degrade quinoline. However, the key bacteria, which open the food box for quinoline degradation, did not directly benefit from the hydroxylation under limited oxygen condition.

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References

1. Reineke AK, Göen T, Preiss A, Hollender J. 2007. Quinoline, derivatives at a tar oil contaminated site: hydroxylated products as indicator for natural attenuation? Environmental Science & Technology, 41(15):5314.
2. Hirao K, Shinohara Y, Tsuda H, Fukushima S, Takahashi M. 1976. Carcinogenic activity of quinoline on rat liver. Cancer Research, 36(2 Pt 1):329.
3. Nagao M, Yahagi T, Seino Y, Sugimura T, Ito N. 1977. Mutagenicities of quinoline and its derivatives. Mutation Research, 42(3):335-341.
4. Ramirez I, Volcke EIP, Rajinikanth R, Steyer JP. 2009. Modeling microbial diversity in anaerobic digestion through an extended ADM1 model. Water Research, 43:2787-2800.

5. Sun Q, Bai Y, Zhao C, Xiao Y, Wen D, Tang X. 2009. Aerobic biodegradation characteristics and metabolic products of quinoline by a *Pseudomonas* strain. Bioresource Technology, 100(21): 5030-5036.
6. Bai Y, Sun Q, Zhao C, Wen D, Tang X. 2010. Quinoline biodegradation and its nitrogen transformation pathway by a *Pseudomonas* sp. strain. Biodegradation, 21(3):335-344.
7. Qiao L, Wang J. 2010. Biodegradation characteristics of quinoline by *Pseudomonas putida*. Bioresource Technology, 101(19):7683-7686.
8. Zhu S, Liu Q, Fan L, Ni J. 2008. Degradation of quinoline by *Rhodococcus* sp. QL2 isolated from activated sludge. Journal of Hazardous Materials, 160(2-3):289-294.
9. Tuo B, Yan J, Fan B, Yang Z, Liu J. 2012. Biodegradation characteristics and bioaugmentation potential of a novel quinoline-degrading strain of *Bacillus* sp. isolated from petroleum-contaminated soil. Bioresource Technology, 107(3):55-60.
10. Felczak A, Zawadzka K, Lisowska K. 2014. Efficient biodegradation of quinoline – Factors determining the process. International Biodeterioration & Biodegradation 96 127-134.
11. Li Y, Wang L, Liao L, Sun L, Zheng G, Luan J, Gu G. 2010. Nitrate-dependent biodegradation of quinoline, isoquinoline, and 2-methylquinoline by acclimated activated sludge. J Hazard Mater. 173(1-3), 151-158. doi: 10.1016/j.jhazmat.2009.08.061.
12. Bai Y, Sun Q, Xing R, Wen D, Tang X. 2011. Analysis of denitrifier community in a bioaugmented sequencing batch reactor for the treatment of coking wastewater containing pyridine and quinoline. Appl Microbiol Biotechnol. 90(4), 1485-1492. doi: 10.1007/s00253-011-3139-0.
13. Mao Y, Zhang X, Yan X, Liu B, Zhao L. 2008. Development of group-specific PCR-DGGE fingerprinting for monitoring structural changes of *Thauera* spp. in an industrial wastewater treatment plant responding to operational perturbations. J Microbiol Methods 75(2), 231-236.
14. Mao Y, Zhang X, Xia X, Zhong H, Zhao L. 2010. Versatile aromatic compound-degrading capacity and microdiversity of *Thauera* strains isolated from a coking wastewater treatment bioreactor. J Ind Microbiol Biotechnol 37(9), 927-934.
15. Zhang X, Yue S, Zhong H, Hua W, Chen R, Cao Y, Zhao L. 2011. A diverse bacterial community in an anoxic quinoline-degrading bioreactor determined by using pyrosequencing and clone library analysis. Appl Microbiol Biotechnol 91(2), 425-434.
16. Wang Y, Tian H, Huang F, Long W, Zhang Q, Wang J, Zhu Y, Wu X, Chen G, Zhao L, Bakken LR, Frostegård Å, Zhang X. 2017. Time-resolved analysis of a denitrifying bacterial community revealed a core microbiome responsible for the anaerobic degradation of quinoline. Scientific Reports, 7(1), 14778.
17. Licht D, Johansen SS, Arvin E, Ahring BK. 1997. Transformation of indole and quinoline by *Desulfobacterium indolicum* (DSM 3383). Applied Microbiology & Biotechnology 47(2) 167-172.
18. Liu B, Zhang F, Feng X, Liu Y, Yan X, Zhang X, Wang L, Zhao L. 2006. *Thauera* and *Azoarcus* as functionally important genera in a denitrifying quinoline-removal bioreactor as revealed by microbial community structure comparison. FEMS Microbiol Ecol 55(2) 274-286.
19. Paulin MM, Nicolaisen MH, Jacobsen CS, Gimsing AL, Sørensen J, Bælum J. 2013. Improving Griffith's protocol for co-extraction of microbial DNA and RNA in adsorptive soils. Soil Biol. Biochem. 63 37-49.

20. Zhang Q, Wu Y, Wang J, Wu G, Long W, Xue Z, Wang L, Zhang X, Pang X, Zhao Y, Zhao L, Zhang C. 2016. Accelerated dysbiosis of gut microbiota during aggravation of DSS-induced colitis by a butyrate-producing bacterium. Sci. Rep. 6: 27572.
21. Edgar RC. 2013. UPARSE: highly accurate OTU sequences from microbial amplicon reads. Nat. Methods 10:996-1000.
22. Edgar RC, Haas BJ, Clemente JC, Quince C and Knight R. 2011. UCHIME improves sensitivity and speed of chimera detection. Bioinformatics 27:2194-2200.
23. Cole JR, Wang Q, Fish JA, Chai BL, McGarrell DM, Sun YN, Brown CT, Porras-Alfaro A, Kuske CR, Tiedje JM. 2014. Ribosomal database project: data and tools for high throughput rRNA analysis. Nucleic Acids Res. 42 633-642.
24. Edgar RC. 2010. Search and clustering orders of magnitude faster than BLAST. Bioinformatics 26:2460-2461.
25. Caporaso JG, Kuczynski J, Stombaugh J, Bittinger K, Bushman FD, Costello EK, Fierer N, Peña AG, Goodrich JK, Gordon JI, Huttley GA, Kelley ST, Knights D, Koenig JE, Ley RE, Lozupone CA, McDonald D, Muegge BD, Pirrung M, Reeder J, Sevinsky JR, Turnbaugh PJ, Walters WA, Widmann J, Yatsunenko T, Zaneveld J, Knight R. 2010. QIIME allows analysis of high-throughput community sequencing data. Nat Methods 7:335-336.
26. Kiyohara H, Nagao K, Yana K. 1982. Rapid screen for bacteria degrading water-insoluble, solid hydrocarbons on agar plates. Appl Environ Microbiol. 43(2):454-7.
27. Van der Linde K, Lim BT, Rondeel JMM. 1999. Improved bacteriological surveillance of haemodialysis fluids: a comparison between tryptic soy agar and reasoner's'2A media. Nephrology Dialysis Transplantation 14(10): 2433-2437.
28. Renshaw MA, Olds BP, Jerde CL, Mcveigh MM and Lodge DM. 2015. The room temperature preservation of filtered environmental DNA samples and assimilation into a phenol-chloroform-isoamyl alcohol DNADNA extraction. Molecular Ecology Resources, 15(1):168-176.
29. Wei G, Pan L, Du H, Chen J, Zhao L. 2004. ERIC-PCR fingerprinting-based community DNA hybridization to pinpoint genome-specific fragments as molecular markers to identify and track populations common to healthy human guts. J Microb Method 59(1):91-108.
30. Wu G, Zhang M, Wei C, Wang Y, Yao X, Zhao L, Zhang X. 2016. Draft genome sequence of *Thauera* sp. DTG from a denitrifying quinoline degrading microbial consortium. Applied Environ Biotech 1(1): 38-43.
31. Johansen SS, Arvin E, MosbaK H and Hansen AB. 1997. Degradation pathway of quinolines in a biofilm system under denitrifying conditions. Environ Toxicol Chem 16(9):1821-1828.
32. Mechichi TE, Stackebrandt E,Gad'on N, Fuchs G. 2002. Phylogenetic and metabolic diversity of bacteria degrading aromatic compounds under denitrifying conditions and description of *Thauera phenylacetica* sp. nov. *Thauera aminoaromatica* sp. nov. and *Azoarcus buckelii* sp. nov. Arch Microbiol 178(1):26-35.
33. Bunge M, Lechner U. 2009. Anaerobic reductive dehalogenation of polychlorinated dioxins. Appl Microb Biotech 84(3): 429-444.
34. Rivett DW, Bell T. 2018. Abundance determines the functional role of bacterial phylotypes in complex communities. Nat Microbiol 3(7): 767-772.
35. Großkopf T, Soyer OS. 2014. Synthetic microbial communities. Current Opinion in

519 Microbiology 18(4): 72-77.
520 36. Holton G. 1977. Sociobiology: the new synthesis? Newsletter on Science Technology &
521 Human Values 46(21):28-43.
522 37. Godsy EM, Goerlitz DF, Grbic-Galic D. 1992. Methanogenic biodegradation of e Ground
523 Water 20: 232-242.
524 38. Lu X, Zhen G, Estrada AL, Chen M, Ni J. Hojo T. 2015. Operation performance and granule
525 characterization of upflow anaerobic sludge blanket (UASB) reactor treating wastewater with
526 starch as the sole carbon source. Bioresour Technol. 180: 264-273.
527 39. Arfmann H, Timmis KN, Wittich R. 1997. Mineralization of 4-chlorodibenzofuran by a
528 consortium consisting of *sphingomonas* sp. strain RW1 and *Burkholderia* sp. strain
529 JWS. Appl Environ Microbiol 63(9): 3458-62.
530 40. Gilbert ES, Walker AW, Keasling JD. 2003. A constructed microbial consortium for
531 biodegradation of the organophosphorus insecticide parathion. Appl Microb Biotech 61(1):
532 77-81.
533 41. Pelz O, Tesar M, Wittich RM, Moore ERB, Timmis KN, Abraham WR. 1999. Towards
534 elucidation of microbial community metabolic pathways: unravelling the network of carbon
535 sharing in a pollutant- - degrading bacterial consortium by immunocapture and isotopic ratio
536 mass spectrometry. Environ Microbiol 1(2):167-174.

Figure legends

Figure 1. The abundance of six most predominant OTUs among 460 OTUs from the quinoline-degrading bioreactor.

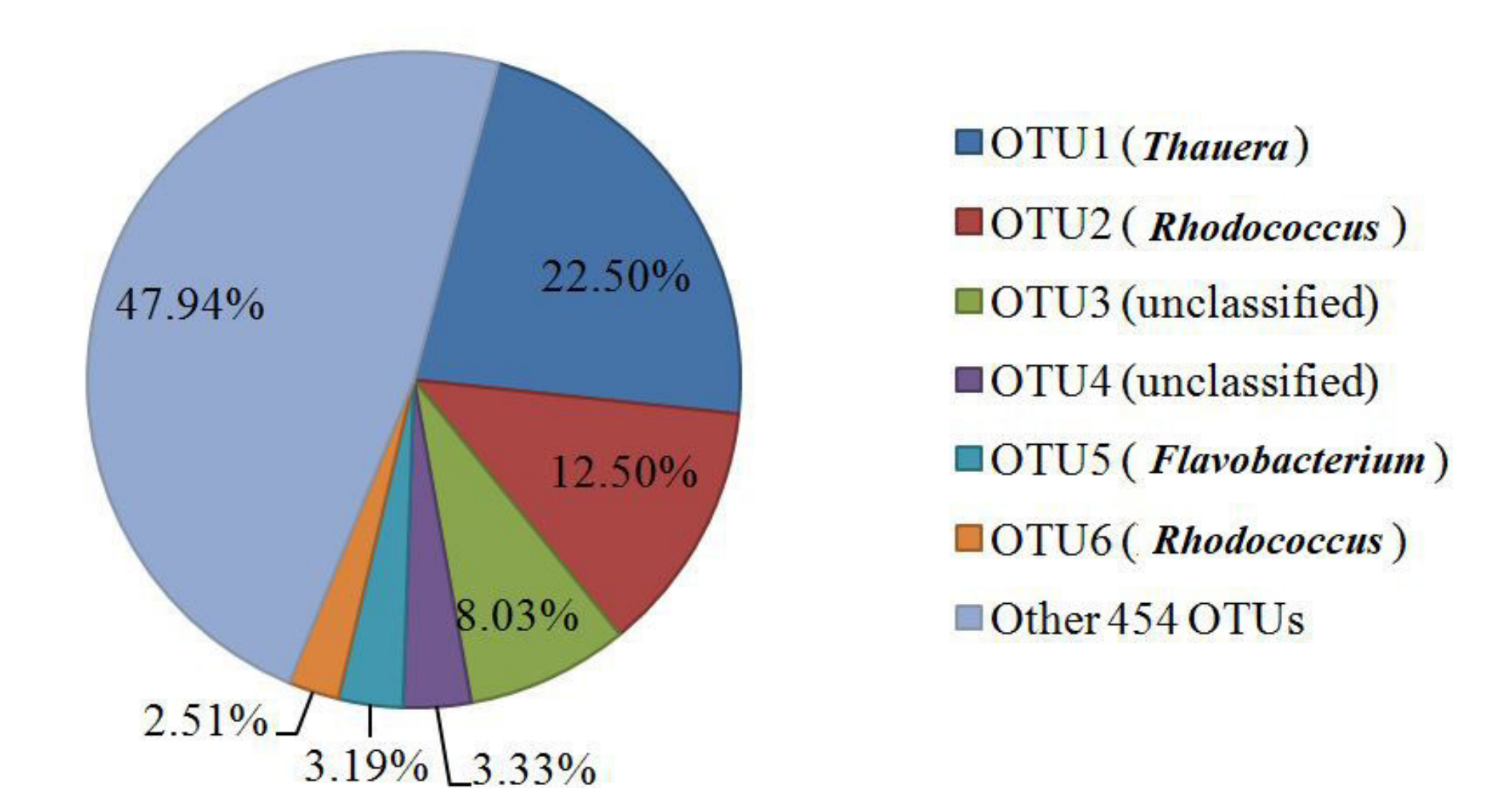
Figure 2. Phylogenetic tree based on the 16S rRNA gene sequences of isolates from quinoline degrading bioreactor and other related *Thauera* species from NCBI database. Numbers in bracket are the sequences accession number in GenBank. The bootstrap values of Neighbor-Joining analysis were labeled at the nodes. Diamond label means isolates in this study; square label means isolates from same reactor in previous study.

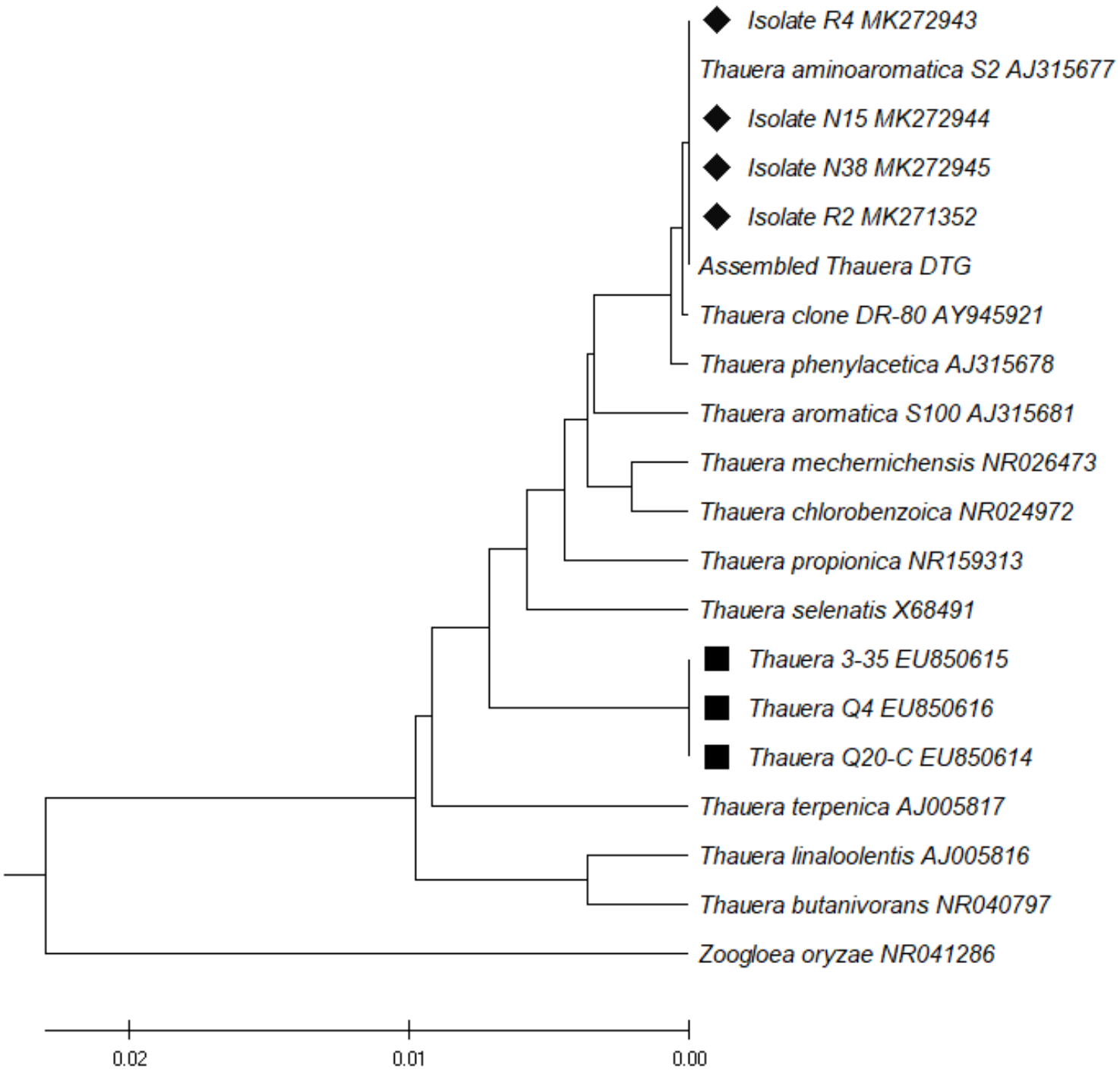
Figure 3. Dynamics of 2-hydroxyquinoline (2-OH-Q) and nitrate removal by four *Thauera* strains. a) degradation of 50 mg/L 2-OH-Q and 1mM nitrate degradation; b) the performance of 30 mg/L 2-OH-Q degradation under different pH conditions. SC represents the sterile control.

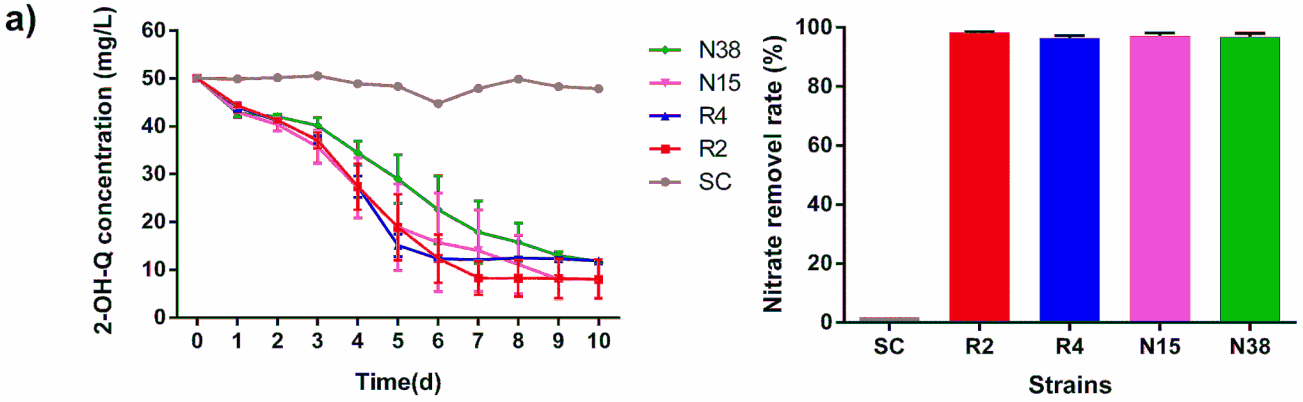
Figure 4. Transformation of quinoline by *Rhodococcus pyridinivorans* YF3 under different oxygen availability a) Concentration of quinoline and 2-hydroxyquinoline (2-OH-Q); b) The proportion of different components remained in cultures.

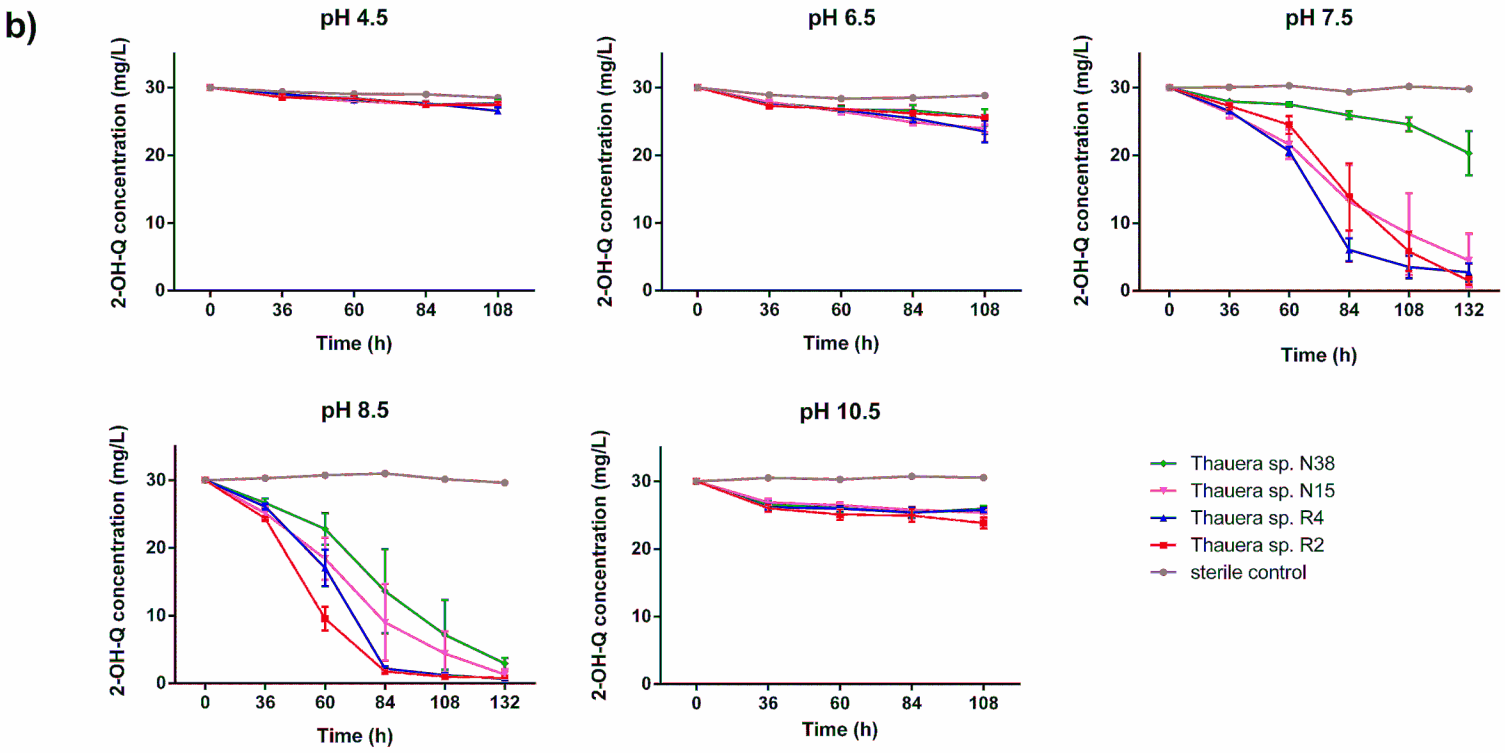
Figure 5. Dynamics of quinoline degradation by the co-culture of *Rhodococcus* sp. YF3 and *Thauera* sp. R2. a） under limited oxygen supplement with the inoculum cultured in NB medium; different colors represent different oxygen supplied conditions. b) under anaerobic condition with the inoculum precultured aerobically in medium containning quinoline. Blue line represents *Rhodococcus* sp. YF3 alone, while red line represents co-culture of *Rhodococcus* sp. YF3 and *Thauera* sp. R2. Solid line in a) and b) represents the change of quinoline concentration while the dotted line represent the change of 2-hydroxyquinoline (2-OH-Q) concentration.

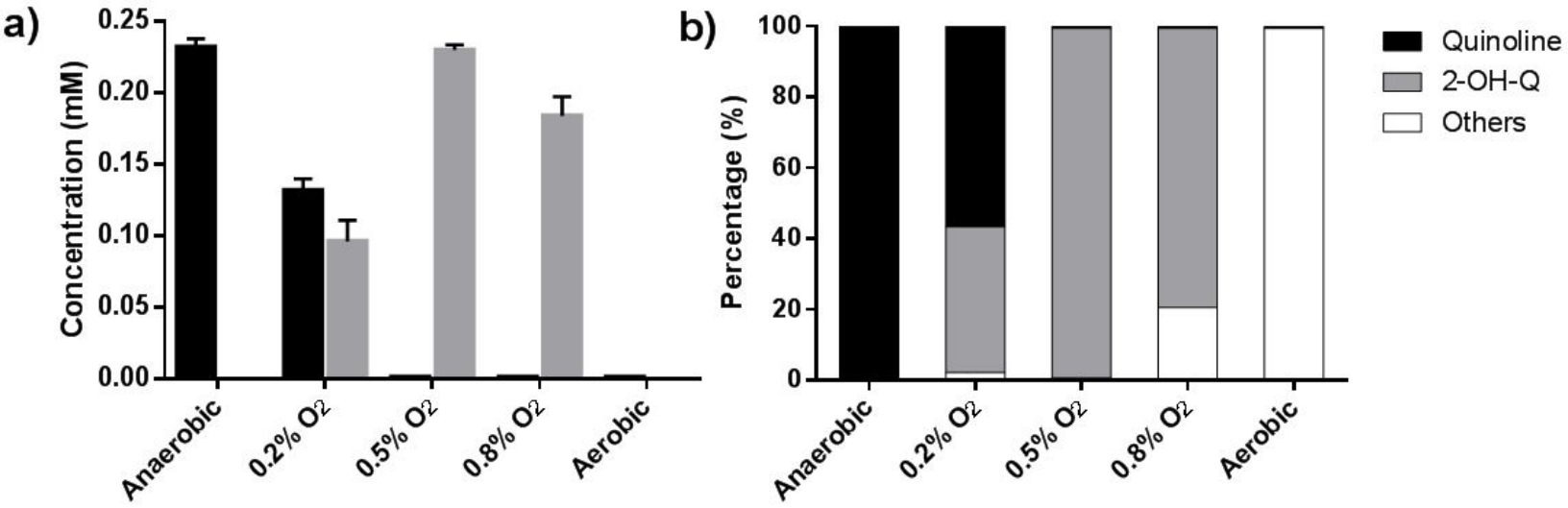
Figure 6. Proposed model for a syntrophic degradation of quinoline by *Rhodococcus* sp. YF3 and *Thauera* sp. R2.

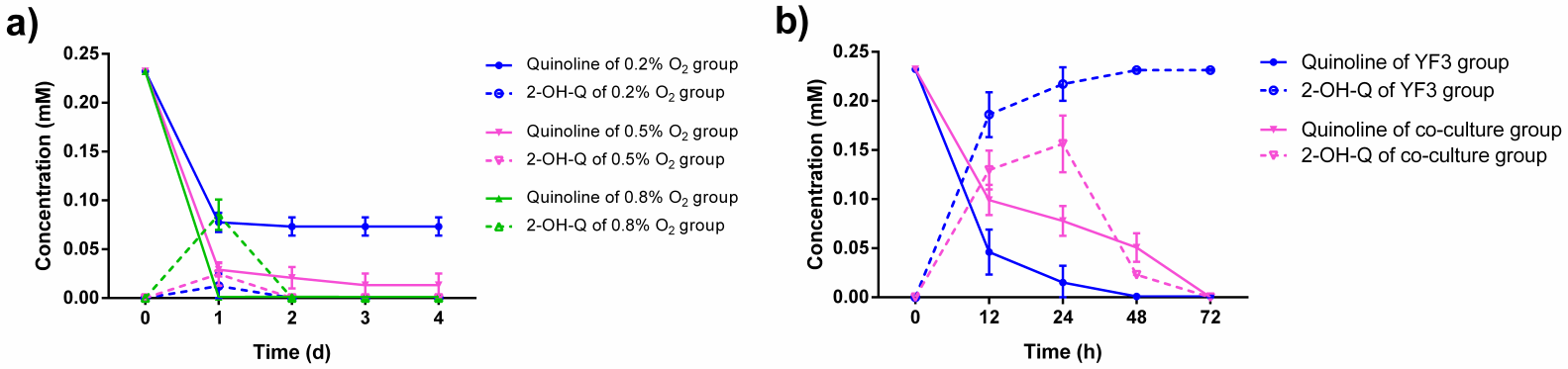

47.94%
22.50%
12.50%
8.03%
2.51%
3.19%
3.33%
OTU1 (Thauera)
OTU2 (Rhodococcus)
OTU3 (unclassified)
OTU4 (unclassified)
OTU5 (Flavobacterium)
OTU6 (Rhodococcus)
Other 454 OTUs

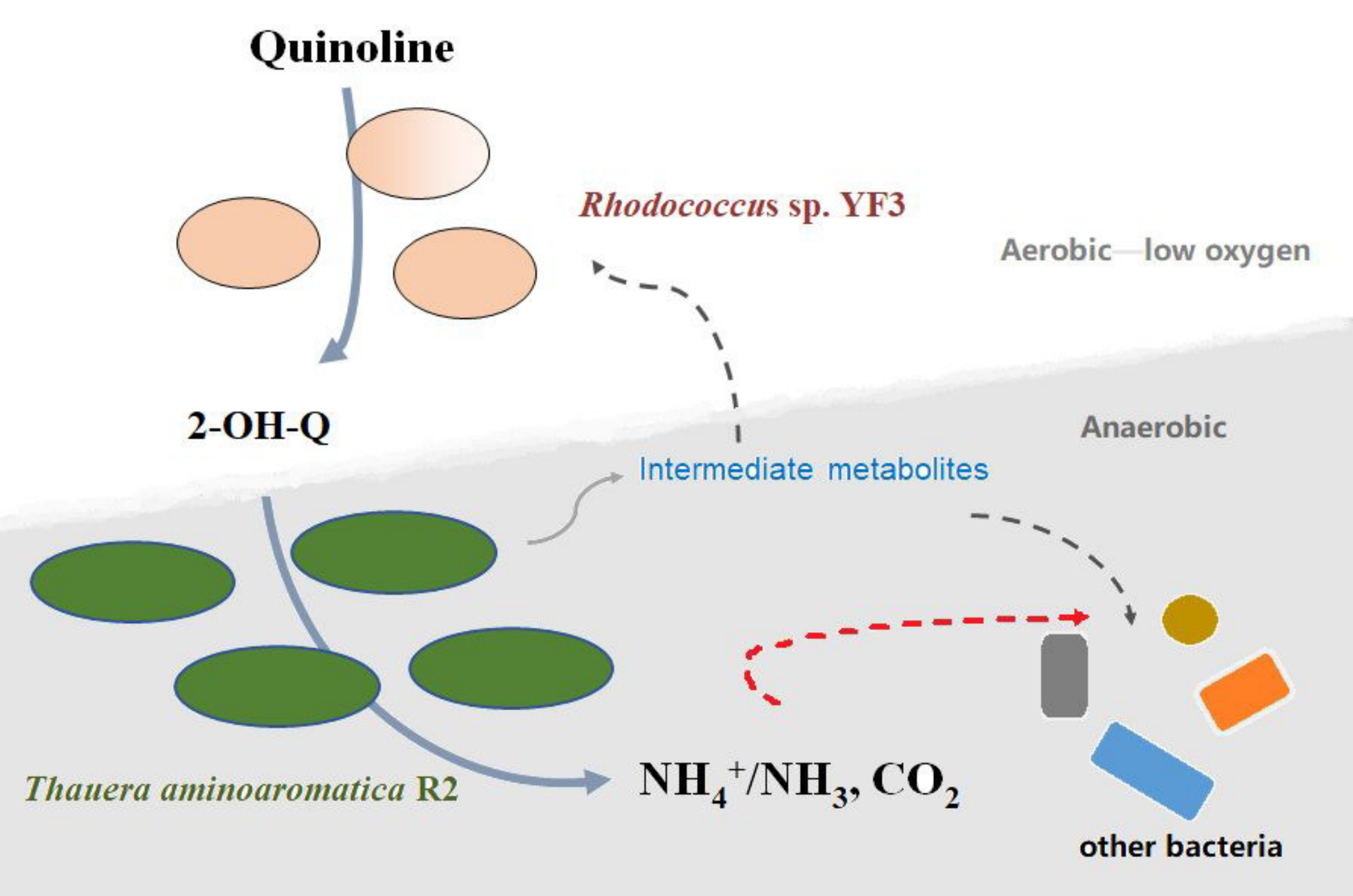

Isolate R4 MK272943
Thauera aminoaromatica S2 AJ315677
Isolate N15 MK272944
Isolate N38 MK272945
Isolate R2 MK271352
Assembled Thauera DTG
Thauera clone DR-80 AY945921
Thauera phenylacetica AJ315678
Thauera aromatica S100 AJ315681
Thauera mechernichensis NR026473
Thauera chlorobenzoica NR024972
Thauera propionica NR159313
Thauera selenatis X68491
Thauera 3-35 EU850615
Thauera Q4 EU850616
Thauera Q20-C EU850614
Thauera terpenica AJ005817
Thauera linaloolentis AJ005816
Thauera butanivorans NR040797
Zoogloea oryzae NR041286
0.02
0.01
0.00


a)
2-OH-Q concentration (mg/L)
Time(d)
N38
N15
R4
R2
SC
Nitrate removel rate (%)
Strains
SC
R2
R4
N15
N38


b)
pH 4.5
pH 6.5
pH 7.5
pH 8.5
pH 10.5
2-OH-Q concentration (mg/L)
Time (h)
Thauera sp. N38
Thauera sp. N15
Thauera sp. R4
Thauera sp. R2
sterile control


a)
Concentration (mM)
0.25
0.20
0.15
0.10
0.05
0.00
Anaerobic
0.2% O2
0.5% O2
0.8% O2
Aerobic
b)
Percentage (%)
100
80
60
40
20
0
Anaerobic
0.2% O2
0.5% O2
0.8% O2
Aerobic
Quinoline
2-OH-Q
Others


a)
Concentration (mM)
0.25
0.20
0.15
0.10
0.05
0.00
0
1
2
3
4
Time (d)
Quinoline of 0.2% O2 group
2-OH-Q of 0.2% O2 group
Quinoline of 0.5% O2 group
2-OH-Q of 0.5% O2 group
Quinoline of 0.8% O2 group
2-OH-Q of 0.8% O2 group
b)
Concentration (mM)
0.25
0.20
0.15
0.10
0.05
0.00
0
12
24
48
72
Time (h)
Quinoline of YF3 group
2-OH-Q of YF3 group
Quinoline of co-culture group
2-OH-Q of co-culture group


Quinoline
Rhodococcus sp. YF3
Aerobic—low oxygen
2-OH-Q
Anaerobic
Intermediate metabolites
Thauera aminoaromatica R2
NH4+/NH3, CO2
other bacteria