



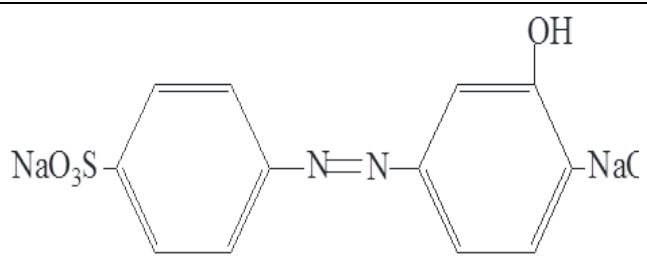
## Supplementary Material

# Structure-Based Long-Term Biodegradation of the Azo Dye: Insights from the Bacterial Community Succession and Efficiency Comparison

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**Table S1.** Chemical characteristics of inspected azo dyes.

| Name                          | Molecular weight | Chemical structure | $\lambda_{\text{max}}$ |
|-------------------------------|------------------|--------------------|------------------------|
| Methyl orange                 | 327              |                    | 484                    |
| Methyl red                    | 269              |                    | 484                    |
| Orange I                      | 350              |                    | 484                    |
| Orange G                      | 452              |                    | 480                    |
| Tartrazine                    | 534              |                    | 424                    |
| Alizarin yellow R sodium salt | 309              |                    | 456                    |

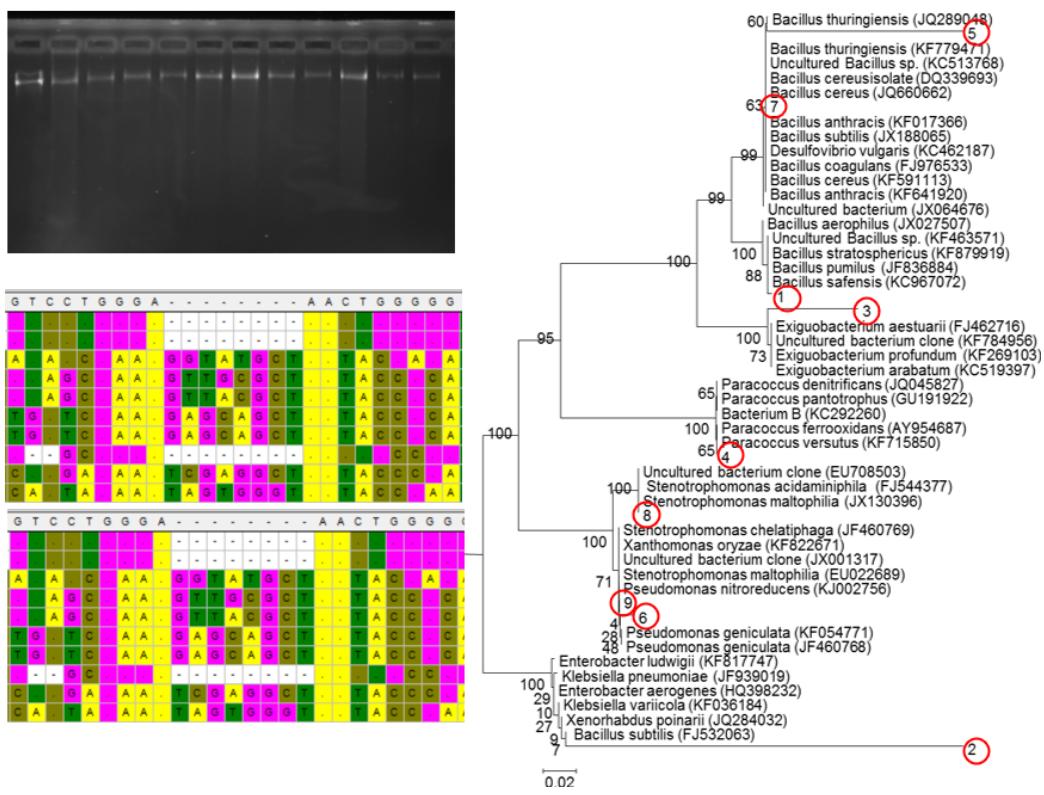


**Table S2.** Features of isolated strains from acclimated activated sludge.

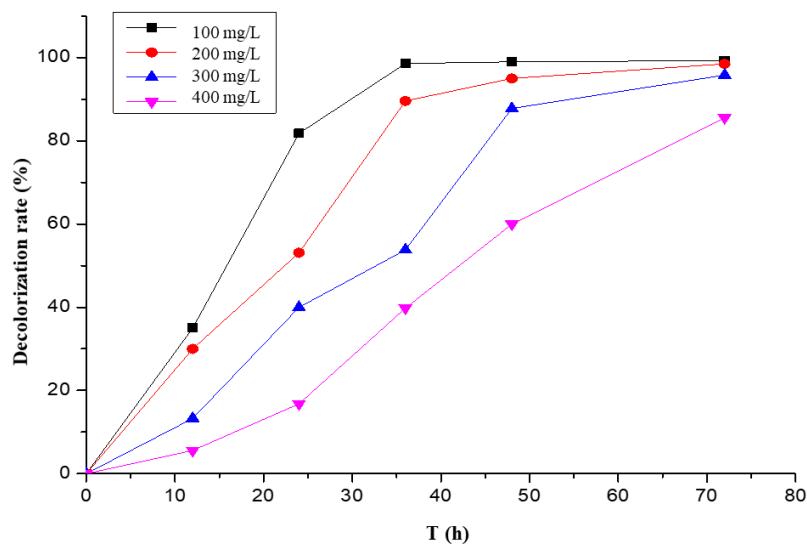
| Colony no. | Strain (access No. in NCBI and % similarity)                   | Thickness        | Size       | Density | Surfaces | Edge        | Shape      | Color difference between up and down side of the colony | Water soluble pigment | Transparency         |
|------------|--|------------------|------------|---------|----------|-------------|------------|---|-----------------------|----------------------|
| T-1        | <i>Bacillus aerophilus</i> strain KG-J-2(JX027507, 97%)        | relatively thick | 1~1.8 mm   |         | Rough    | Wavy        | Curley     | Different   | Pink                  | —                    |
| T-2        | <i>Klebsiella variicola</i> strain RVEV3(KF03618 4, 97%)       | Thin             | 1~1.3 mm   |         | Smooth   | Neat        | Flat       | Different   | Red                   | —                    |
| T-3        | <i>Exiguobacterium aestuarii</i> strain YS6 (FJ462716, 90)     | Thin             | 1.2~1.5 mm |         | Smooth   | Neat        | Flat       | Different   | White                 | —                    |
| T-4        | <i>Paracoccus verus</i> strain NMD4 (KF715850, 99)             | relatively thick | 0.8~1.2 mm |         | Smooth   | Neat        | Low convex | Same  | Colorless             | Slightly transparent |
| T-5        | <i>Bacillus anthracis</i> strain WY2 (KF641920, 97%)           | relatively thick | 1.2~2 mm   | Loose   | Rough    | Round teeth | Low convex | Same  | Colorless             | —                    |
| T-6        | <i>Pseudomonas geniculata</i> strain Ka38 (JF460769, 96%)      | relatively thick | 0.8~1.5 mm |         | Smooth   | Neat        | Papillary  | Different   | Yellow                | —                    |
| T-7        | <i>Bacillus cereus</i> strain BVC79 (JQ660662, 99%)            | relatively thick | 0.8~1.2 mm |         | Smooth   | Teeth       | Predentary | Same  | Colorless             | Slightly transparent |
| T-8        | <i>Stenotrophomonas maltophilia</i> strain T11 (JX130396, 99%) | Thin             | 1.0~1.2 mm |         | Smooth   | Neat        | Flat       | Different   | Light yellow          | —                    |
| T-9        | <i>Pseudomonas nitroreducens</i> strain LH3 (KJ002756, 99%)    | Thin             | 1.0~1.2 mm |         | Smooth   | Irregular   | Flat       | Different   | White                 | —                    |

**Table S3.** Physiological-biochemical characteristics of isolated strains.

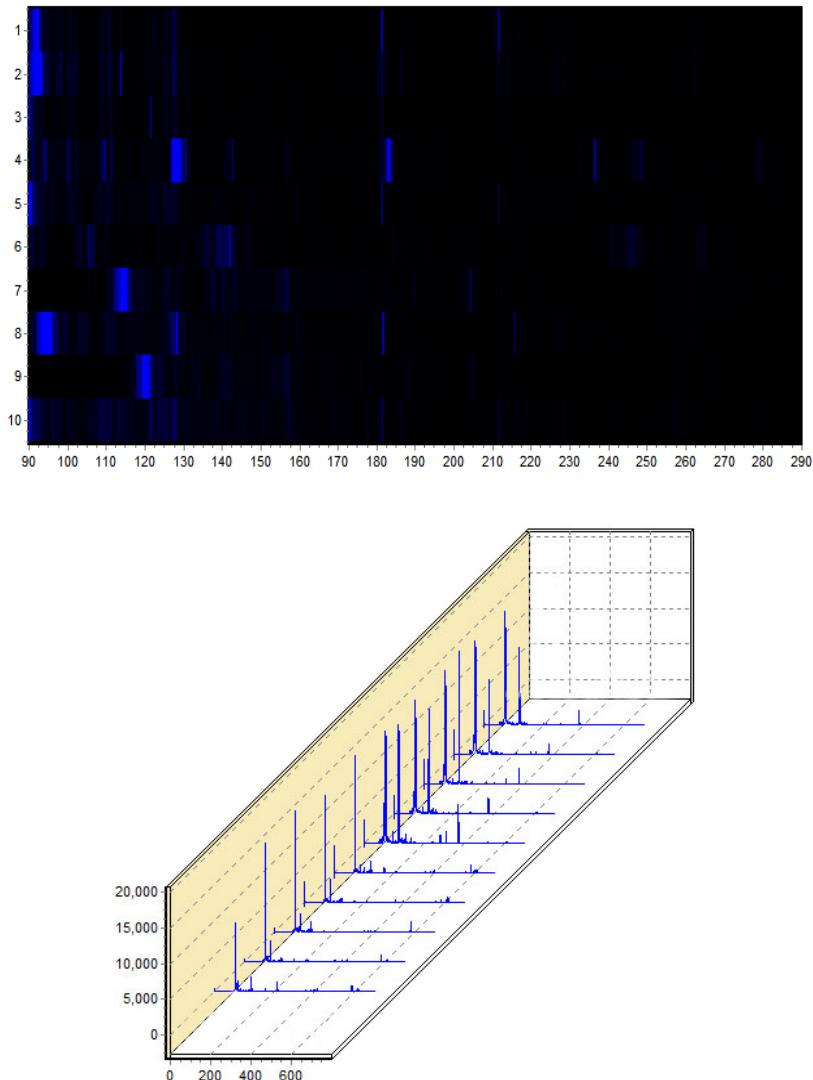
| Title                   | T-1 | T-2 | T-3 | T-4 | T-5 | T-6 | T-7 | T-8 | T9 | Result |
|-------------------------|-----|-----|-----|-----|-----|-----|-----|-----|----|--------|
| Gram stain              | +   | -   | +   | -   | +   | -   | +   | -   | -  | -      |
| Catalase                | +   | +   | +   | +   | +   | +   | +   | +   | +  | +      |
| Nitrate reduction       | +   | +   | +   | +   | +   | +   | +   | +   | +  | +      |
| Starch hydrolysis       | +   | +   | -   | -   | +   | +   | +   | -   | -  | -      |
| Liquefaction of gelatin | +   | +   | +   | -   | +   | +   | +   | +   | +  | +      |
| Urease                  | -   | +   | +   | +   | -   | +   | -   | -   | -  | -      |
| Arginine hydrolase      | -   | -   | +   | +   | -   | +   | -   | +   | +  | +      |
| Arginine decarboxylase  | +   | +   | +   | +   | +   | +   | +   | +   | +  | +      |
| Citrate                 | +   | +   | +   | +   | +   | +   | +   | -   | -  | -      |
| 45 °C growth            | -   | -   | +   | -   | -   | -   | -   | -   | -  | -      |



**Figure S1.** Molecular identification.



**Figure S2.** The concentration effect on the percentage (%) decolorization of methyl orange in the dewatered sludge.



**Figure S3.** The metagenomic DNA gel electrophoresis patterns of different samples.

**Table S4.** The diversity index of the bacterial communities of different treatments.<sup>[1]</sup>

| Type                          | TRLFP unit* | SIMPSON(J) | SHAN-NON(H) | EVENESS | BRILLOUIN | McIntosh (Dmc) |
|-------------------------------|-------------|------------|-------------|---------|-----------|----------------|
| Methyl orange                 | 716478      | 0.7271     | 2.5951      | 0.6223  | 2.5949    | 0.4781         |
| Methyl red                    | 636292      | 0.5578     | 1.6692      | 0.5946  | 1.6692    | 0.3355         |
| Orange I                      | 651797      | 0.6422     | 1.8923      | 0.6761  | 1.7812    | 0.3698         |
| Orange G                      | 698617      | 0.6838     | 2.0092      | 0.7157  | 2.0092    | 0.4382         |
| Tartrazine                    | 714004      | 0.6785     | 2.0754      | 0.7393  | 2.0753    | 0.4335         |
| Alizarin yellow R sodium salt | 589331      | 0.6998     | 2.1163      | 0.7538  | 2.1162    | 0.4526         |
| Azo dye mixture               | 659168      | 0.7294     | 2.2828      | 0.7609  | 2.2827    | 0.4804         |
| Raw dewatered sludge          | 1271198     | 0.9107     | 3.0251      | 0.9125  | 3.2141    | 0.5903         |

\* TRLFP unit is the basic potential taxa information based on the specific restriction sequence information.

**Table S5.** The full names of species.

| Serial number | Name                |
|---------------|---------------------|
| Sp1           | Abiotrophia         |
| Sp2           | Acidimicrobium      |
| Sp3           | Acromatium          |
| Sp4           | Aeromonas           |
| Sp5           | Alicyclobacillus    |
| Sp6           | Anaerobranca        |
| Sp7           | Aneurinibacillus    |
| Sp8           | Anhibacillus        |
| Sp9           | Arhodomonas         |
| Sp10          | Bdellovibrio        |
| Sp11          | Beggiatoa           |
| Sp12          | Brevibacillus       |
| Sp13          | Brevibacterium      |
| Sp14          | Buchnera            |
| Sp15          | Burkholderia        |
| Sp16          | Caloramator         |
| Sp17          | Capnocytophaga      |
| Sp18          | Caryophanon         |
| Sp19          | Chlorobium          |
| Sp20          | Citrobacter         |
| Sp21          | Clavibacter         |
| Sp22          | Clostridium         |
| Sp23          | Cytophaga           |
| Sp24          | Desulfitibacterium  |
| Sp25          | Dictyoglomus        |
| Sp26          | Ectothiorthodospira |
| Sp27          | Empedobacter        |
| Sp28          | Endosymbriout       |
| Sp29          | Escherichia         |
| Sp30          | Eubacterium         |
| Sp31          | Fibrobacter         |
| Sp32          | Flavabacterium      |
| Sp33          | Flexibacter         |
| Sp34          | Francisella         |
| Sp35          | Frankia             |

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|      |                            |
|------|----------------------------|
| Sp36 | Fusobacterium              |
| Sp37 | Gemmata                    |
| Sp38 | Geobacter                  |
| Sp39 | Halobacillus               |
| Sp40 | Halomonas                  |
| Sp41 | Kurthia                    |
| Sp42 | Lactobacillus              |
| Sp43 | Legionella                 |
| Sp44 | Leptothrix                 |
| Sp45 | Leuconostoc                |
| Sp46 | Luteococcus                |
| Sp47 | Macroccoccus               |
| Sp48 | Mannheimia                 |
| Sp49 | Marinobacter               |
| Sp50 | Methylocaldum              |
| Sp51 | Methylosinus               |
| Sp52 | Methlophilus               |
| Sp53 | Methylarcula(Methyoarcula) |
| Sp54 | Microbacterium             |
| Sp55 | Microcystis                |
| Sp56 | Microscilla                |
| Sp57 | Mycoplasma                 |
| Sp58 | Myxiococcus                |
| Sp59 | Oceanospirillum            |
| Sp60 | Oerskovia                  |
| Sp61 | Omithobacterium            |
| Sp62 | Ornithobacterium           |
| Sp63 | Paenibacillus              |
| Sp64 | Pasteurella                |
| Sp65 | Pectinatus                 |
| Sp66 | Phaeospirillum             |
| Sp67 | Phlomobacter               |
| Sp68 | Phlomobacter               |
| Sp69 | Photobacterium             |
| Sp70 | Phytoplasma                |
| Sp71 | Planococcus                |
| Sp72 | Porphyromonas              |
| Sp73 | Prevotella                 |
| Sp74 | Proteus                    |
| Sp75 | Psychroserpens             |
| Sp76 | Quinella                   |
| Sp77 | Rathayibacter              |
| Sp78 | Rhizobium                  |
| Sp79 | Rhodopila                  |
| Sp80 | Rikenella                  |
| Sp81 | Rubrivivax                 |
| Sp82 | Rubrobacter                |
| Sp83 | Saccharococcus             |
| Sp84 | Saccharothrix              |
| Sp85 | Salmonella                 |
| Sp86 | Secale                     |
| Sp87 | Selenomonas                |

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|       |                    |
|-------|--------------------|
| Sp88  | Shewanella         |
| Sp89  | Spiroplasma        |
| Sp90  | Sporolactobacillus |
| Sp91  | Sporosarcina       |
| Sp92  | Springomonas       |
| Sp93  | Stretomyces        |
| Sp94  | Sulfobacillus      |
| Sp95  | Synechococcus      |
| Sp96  | Telluria           |
| Sp97  | Therinomonopora    |
| Sp98  | Thermosiphon       |
| Sp99  | Treponema          |
| Sp100 | Vagococcus         |
| Sp101 | Xanthomonas        |
| Sp102 | Xylella            |
| Sp103 | Zea mays           |
| Sp104 | Zymophilus         |

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**Reference:**

- 1 Barinova, S.; Tavassi, M. Study of seasonal influences on algal biodiversity in the River Yarqon (central Israel) by bio-indication and canonical correspondence analysis (CCA). *Turk. J. Botany*, **2009**, *33*, 353–372, doi:10.3906/bot-0812-12.