

## ***Supplementary Material A***

### **A.1 Development of the biodegradation assays for azo dyes and whitener**

First, the influence of centrifugation on sedimentation of dyes was tested by determining the absorption or emission intensity of the dyes before and after centrifugation (12,000×g, 5 min). For whitener, excitation at 315 nm showed a maximum wavelength emission at 440 nm, as for 4,4'-diamino-2,2'-stilbenedisulfonic acid (DSDS), and the coefficient between the fluorescence intensity after and before centrifugation ( $I_{\text{cent}}/I$ ) was 98%. For black dye an absorption decrease of 4% at both peak maxima was recorded, for red, blue and direct blue 15 dye (DB15) no decrease in absorption was detected and yellow dye showed a decrease of 50%. Calibration curves for all tests in liquid media were generated for molar concentrations at  $\lambda_{\text{max}}$  and the volume of liquid in microplates was kept constant to ensure accurate absorbance measurements. To quantitatively determine the amount of unhydrolyzed dye by measuring the absorption of supernatants, the tests with NB in microplates had to be completed in a maximum of three days. To determine the activities of the most active bacteria, initially, relative standard error between the triplicates was maximum 3%. During incubation the microtiter plates were covered with sterile lids and were packed in bags to prevent evaporation. Wet paper was inserted into the bags to maintain a constant humidity.

### **References**

- Hočevár, B., Grilc, M., and Likozar, B. (2019). Aqueous Dehydration, Hydrogenation, and Hydrodeoxygenation Reactions of Bio-Based Mucic Acid over Ni, NiMo, Pt, Rh, and Ru on Neutral or Acidic Catalyst Supports. *Catalysts*, 9, 286-306. <https://doi:10.3390/catal9030286>
- Marshall, C. J., and Kunkle, C. A. (1992). Mineral dye pigments. U.S. Patent No 5,106, 421.

**Supplementary Table A.1** Whitewater analysis of the wood-free paper mill within a period of two months with average, minimum and maximum values.

<b>t (day)<sup>a</sup></b>	<b>COD (mg/L)</b>	<b>DOC (mg/L)</b>	<b>BOD<sub>5</sub> (mg/L)</b>	<b>HCO<sub>3</sub><sup>-</sup> (mg/L)</b>	<b>Cl<sup>-</sup> (mg/L)</b>	<b>NO<sub>3</sub><sup>-</sup> (mg/L)</b>	<b>SO<sub>4</sub><sup>2-</sup> (mg/L)</b>	<b>Na<sup>+</sup> (mg/L)</b>	<b>K<sup>+</sup> (mg/L)</b>	<b>NH<sub>4</sub><sup>+</sup> (mg/L)</b>	<b>Ca<sup>2+</sup> (mg/L)</b>	<b>Mg<sup>2+</sup> (mg/L)</b>	<b>pH</b>
<b>AVG</b>	<b>303</b>	<b>94</b>	<b>166</b>	<b>153</b>	<b>40</b>	<b>8</b>	<b>179</b>	<b>78</b>	<b>0</b>	<b>2</b>	<b>59</b>	<b>15</b>	<b>7.5</b>
<b>MIN</b>	<b>190</b>	<b>31</b>	<b>80</b>	<b>50</b>	<b>25</b>	<b>1.6</b>	<b>109</b>	<b>62</b>	<b>0</b>	<b>1.7</b>	<b>41</b>	<b>10</b>	<b>6.8</b>
<b>MAX</b>	<b>500</b>	<b>175</b>	<b>254</b>	<b>269</b>	<b>62</b>	<b>15</b>	<b>309</b>	<b>95</b>	<b>1.5</b>	<b>3.5</b>	<b>88</b>	<b>29</b>	<b>8.2</b>
<b>-1</b>	232	134	158	165	49	7.8	183	78	0	2.5	52	16	7
<b>1</b>	214	54		130	34	15	175	62	0		54	16	7.1
<b>2</b>	278	52	80	114	25	13	309	67	0		88	17	7.5
<b>5</b>	190	36		111	26	7.4	267	90	0	2	78	13	7.2
<b>6</b>	210	31		90	26	8.4	274	86	0	1.8	79	16	7.5
<b>7</b>	200	37		50	26	9.1	233	70	0		59	14	6.8
<b>8</b>	268	77	109	141	36	9.1	233	95	0	2.2	67	14	7.5
<b>9</b>	320	91		156	45	3.7	132	66	0		43	17	7.3
<b>12</b>	252	78		123	39	5.7	117	66	0	2.5	45	12	7.6
<b>13</b>	356	95		144	36	7.7	156	78	0		52	13	7.6
<b>14</b>	300	120	178	184	46	8.2	125	85	0	2.7	51	13	7.4
<b>15</b>	284	105		180	41	4	138	76	0	1.7	64	14	7.8
<b>16</b>	345	114		170	44	6	109	75	0	2	41	10	7.7
<b>21</b>	362	128		141	36	6.2	150	76	0	2	60	13	7.5
<b>22</b>	500	175	180	168	44	2.3	116	76	0	2.1	50	11	7.3
<b>24</b>	243	74		167	27	4.3	122	76	0		60	12	7.4
<b>28</b>	448	128	183	146	57	13	235	84	0	1.7	61	16	8.2
<b>31</b>	393	120	254	253	52	11.1	171	82	0	2.5	52	14	7.4
<b>59</b>	371	145	183	269	62	1.6	163	87	1.5		71	29	8.2

<sup>a</sup> ... -1 refers to one day before cleaning of the whitewater system and days 1-59 to sampling after restarting of the system.

**Supplementary Table A.2.** Organic additives used as carbon sources with abbreviation, their assumed shares in the industrial wood-free paper fiber slurry, calculated according to production sheets of four different paper grades of a wood-free paper mill and chemical structure with molar mass to calculate the calibration curves; for dyes maximum wavelength absorption or excitation/emission and dye intensity before and after centrifugation at 12,000×g.

Abbr.	Share (%)	Chemical structure
CE	4	Microcrystalline cellulose (VWR, USA) CAS 9004-34-6; M (C <sub>12</sub> H <sub>22</sub> O <sub>11</sub> )=342 g/mol (monomeric unit)
CST	0.4	Cationic potato starch, degree of derivatization 0.04, based on integrals in <sup>1</sup> H-NMR; M(C <sub>6</sub> H <sub>10</sub> O <sub>5</sub> )=162 g/mol (monomeric unit)
RES	0.4	Rosin, CAS No 8050-09-7; contains abietic and pimeric type resin acids; M(C <sub>20</sub> H <sub>30</sub> O <sub>2</sub> )=303 g/mol
AKD	0.18	Alkyl ketene dimers: 2-oxetanone, 3-C12-16-alkyl-4-C13-17-alkylidene derivatives, CAS No 84989-41-3; M=477 g/mol
WH	0.10	Whitening agent; fluorescent brightening agent; tetrasodium 2,2'-ethene-1,2-diylbis[5-({4-[bis(2-hydroxyethyl)amino]-6-[(4-sulfonatophenyl)amino]-1,3,5-triazin-2-yl} amino)benzenesulfonate]; spectral properties similar to 4,4'-diamino-2,2'-stilbenedisulfonic acid; M(C <sub>42</sub> H <sub>44</sub> N <sub>12</sub> Na <sub>4</sub> O <sub>16</sub> S <sub>4</sub> )=1193 g/mol; excitation at 315 nm with maximum emission at 430 nm; I <sub>cent</sub> /I = 98%
NST	0.005	Native soluble starch, CAS 9005-25-8; (2R,SS,4S,5R,6R)-2-(hydroxy methyl)-6-[(2R(xy-oxane-3,4,5-triol,3S,4R,5R,6S)-4,5,6-tri hydroxy-2-(hydroxymethyl)oxan-3-y)oxy-oxane-3,4,5-triol; M(C <sub>6</sub> H <sub>10</sub> O <sub>5</sub> )=162 g/mol (monomeric unit)
ST	/	Starch, soluble (Sigma Aldrich, USA) CAS 9005-84-9; M(C <sub>6</sub> H <sub>10</sub> O <sub>5</sub> )=162 g/mol (monomeric unit)
RED	0.005	Direct red dye 253 (Marshall 1992), CAS 142985-51-1: Dinatrium;(3E)-7-[[4,6-bis(2-hydroxyethylamino)-1,3,5-triazin-2-yl]amino]-4-oxo-3-[[4-[(4-sulfonatophenyl)diazenyl]phenyl] hydrazinylidene] naphthalene-2-sulfonate; M(C <sub>29</sub> H <sub>26</sub> N <sub>10</sub> Na <sub>2</sub> O <sub>9</sub> S <sub>2</sub> )=769 g/mol; λ <sub>max</sub> =510 nm; I <sub>cent</sub> /I = 100%
BLU	0.005	Blue dye, similar biodegradation to direct blue 15, CAS No. 2429-74-5. To evaluate the structural similarity of the dye BLU to DB15, cometabolism tests on 44 selected strains were compared for the biodegradation of BLU and DB15 dye in NB. After 72 h similar discoloration patterns were found. Direct blue 15 is 2,3,3'-[(3,3'-dimethoxy-4,4'-

		biphenylene)bis(azo)]bis [5-amino-4-hydroxy-tetrasodium salt]; $M_{DB15}(C_{34}H_{28}N_6NaO_{16}S_4)=927$ g/mol; $\lambda_{max}$ BLU=560 nm; $\lambda_{max}$ DB15=600 nm; $I_{cent}/I = 100\%$
BLA	0.003	Black dye HM 2482, CAS No. 89857-06-7; 1,3'-bipyridinium,5'-[[4-[[7-[(1',2'-dihydro-6'-hydroxy-3,4-dimethyl-2'-oxo[1,3'-bipyridinium]-5'-yl)azo]-1-hydroxy-3-sulpho-2-naphthalenyl]azo]phenyl]azo]-1'-[3-(dimethylamino)propyl]-1',2'-oxo-, salt with 2-hydroxypropanoic acid; $M(C_{50}H_{57}N_{11}O_{14}S_4)=1068$ g/mol; $\lambda_{max}=440, 590$ nm; $I_{cent}/I = 96\%$
Y	0.003	Yellow dye containing C; H; N; O; S; stilbene derivative; $M^a=180$ g/mol; $\lambda_{max}=400$ nm; $I_{cent}/I = 50\%$
PVA	<0.001	Polyvinyl alcohol, CAS 9002-89-5; $M(C_2H_4O)=44$ g/mol (monomeric unit)
LX	<0.001	Latex dispersion, styrene-butadiene copolymer; $M(C_{12}H_{14})=158$ g/mol (monomeric unit)

<sup>a</sup> Unknown structure, molar weight taken for glucose.

**Supplementary Table A.3.** Protocols with inoculum preparation and composition of media.

Test		Inoculum preparation	Carbon source	Selection media
Biodegradation assays (see Section 2.3)		Colony of the isolate on NB agar was picked and transferred to a circle on selection media. Incubation was at 25 °C for 7-13 days.	starch	M9 10 g/L ST M9 10 g/L CST M9 10 g/L NST
			CE	M9 5 g/L CE
			RES	M9 0.25 g/L RES
			AKD	M9 0.25 g/L AKD
			PVA	M9 5 g/L PVA
			LX	M9 0.25 g/L LX
		Colony of the isolate on NB agar was picked and transferred into M9 media with 47 mg/L of appropriate dye or whitener. After incubation at 25 °C for 3 days 10 µL of the culture was transferred into the selection medium.	BLU	NB + 6 mg/L BLU
			RED	NB + 15 mg/L RED
			BLA	NB + 57 mg/L BLA
			WH	M9 <sub>Glc</sub> + 0.9 g/L WH; supernatant 20-times diluted

		Y	M9 95 mg/L yellow dye and M9 47 mg/L glucose
Time-dependent degradation determination	A colony was transferred into corresponding M9 media with 47 mg/L of appropriate dye or whitener. After incubation at 25 °C for 3 days 10 µL of the culture was transferred into the selection medium.	RED	NB + 13 mg/L RED
		BLU	NB + 10 mg/L BLU
		WH	M9 <sub>Glc</sub> + 0.5 g/L WH; supernatant 20-times diluted
Tests for the repertoire of carbon source usage ( <i>see</i> Section 2.7)	A colony was transferred into M9 <sub>Glc</sub> for 3 days at 25 °C. 10 µL of the culture was transferred into the selection medium.	CST	M9 0.5 g/L CST
		ST	M9 0.5 g/L ST
		AKD	M9 4 g/L AKD
		RED	M9 <sub>Glc</sub> + 5 mg/L RED
		BLU	M9 <sub>Glc</sub> + 5 mg/L BLU
		WH	M9 <sub>Glc</sub> + 5 mg/L WH
		BLU	NB + 5 mg/L BLU
		CE	20 mL / plate; M9 agar with 5 g/L CE
		CST	15 mL / plate, M9 agar with 5 g/L CST
		AKD	20 mL / plate, M9 agar with 0.25 g/L AKD
<i>In vitro</i> co-culturing test ( <i>see</i> Section 2.8)	The growth of the six selected isolates was calibrated with determining cfu vs. OD <sub>600</sub> . Overnight culture was harvested in NB, cleared three times in 9 g/L NaCl. OD <sub>600</sub> was measured and a calibration curve was generated. The amount of volume was adjusted so that an equal initial sum of bacteria per each medium was added, i.e. 10 <sup>7</sup> CFU/mL.	RES	20 mL / plate, M9 agar with 0.25 g/L RES
		synthetic whitewater	M9 (CST, PVA, RES, DB15); in the ratio CST : PVA : RES : DB15 = 1 : 1 : 1 : 0.5
		industrial whitewater	M9 sterile whitewater

Proof of concept pilot test (see Section 2.9)	Separate overnight cultures of the four bacteria of the consortium harvested in NB, cleared in 9 g/L NaCl, OD <sub>600</sub> measured and CFU calculated.	industrial whitewater	whitewater (unsterile) with added urea and H <sub>3</sub> PO <sub>4</sub> in the ratio COD : N : P = 100 : 5 : 1
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**Supplementary Table A.4.** Selected isolates, active for the carbon source which they used during isolation with numbers of isolated bacteria per carbon source and results of biodegradation assays - C/C<sub>0</sub> and OD<sub>600</sub> values and test durations.

Carbon source	Number of isolates	Active isolate	C/C <sub>0</sub> (%)	OD <sub>600</sub> (/) ± RSE(%)	t (d)	C <sub>0</sub> (g/L)
Cationic starch	44	CST37	75	/	9	10
		CST5	83	/		
		CST10	83	/		
		CST16	83	/		
Native starch	31	NST20	83	/	11	10
		NST22	87	/		
Starch, soluble	35	ST12	72	/	13	10
		ST14	0	/		
		ST16	0	/		
Resin	20	RES19	39	/	7	0.25
		RES9	75	/		
		RES13	75	/		
		RES15	75	/		
AKD	27	AKD4	51	/	7	0.25
		AKD5	46	/		
		AKD13	75	/		
		AKD19A	75	/		
PVA	20	PVA19	13	/	7	5
		PVA20	75	/		
		PVA3	75	/		
		PVA7	75	/		
		PVA14	83	/		
Cellulose	22	CE12	0	/	11	5
		CE21	0	/		
		CE7	81	/		
		CE17	46	/		
Latex	23	LX13	31	/	7	0.25
		LX11	75	/		
		LX8	84	/		
Red	20	RED14	0	1.4±4	2	0.015
		RED14A	20	1.5±5		
		RED15A	0	1.0±9		
		RED16B	11	1.7±1		
Blue	27	BLU19	80	0.6±7	0.75	0.006
		BLU23	60	1.6±5		
Whitener	12	WH8	59	0.13±2	3	0.9

Yellow	14	WH5	99	0.04±7	7	0.095
		Y19	50	0.02±0		
		Y17	53	0.02±1		
		Y18	60	0.01±1		
		Y14A	92	0.01±1		
Black	23	BLA14	70	1.8±2	2.8	0.057
		BLA16	70	2.4±3		
		BLA19	84	2.3±3		

**Supplementary Table A.5.** AKD degradation confirmed by decrease in absorption and DOC removal, presented in C/C<sub>0</sub> values; the bacteria were inoculated for 62 h, initially 10<sup>8</sup> cfu/mL and C<sub>0</sub> 4 g/L.

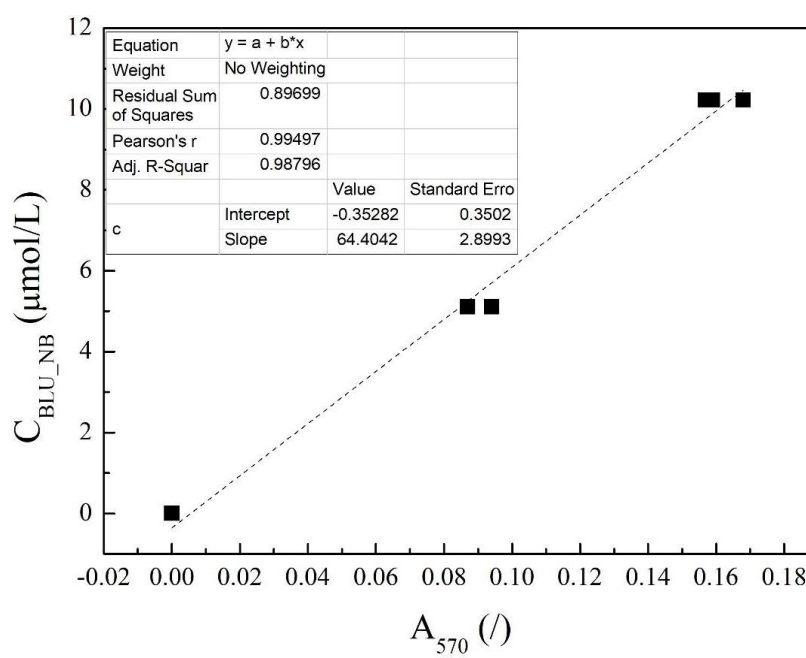
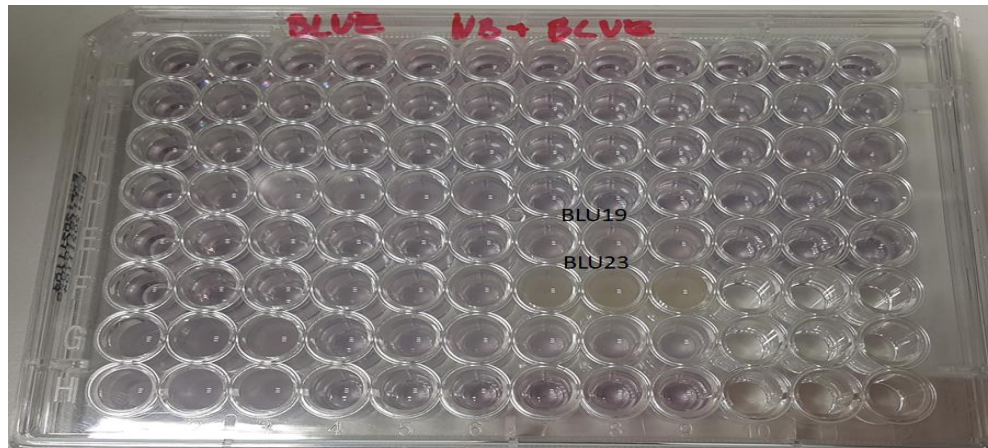
Isolate	C/C <sub>0</sub> (%) ± RSE (%)	
	Absorption	DOC
CST37	56±0	62±0
AKD4	57±0	66±1
RES19	56±0	71±1
BLA14	100±1	100±1



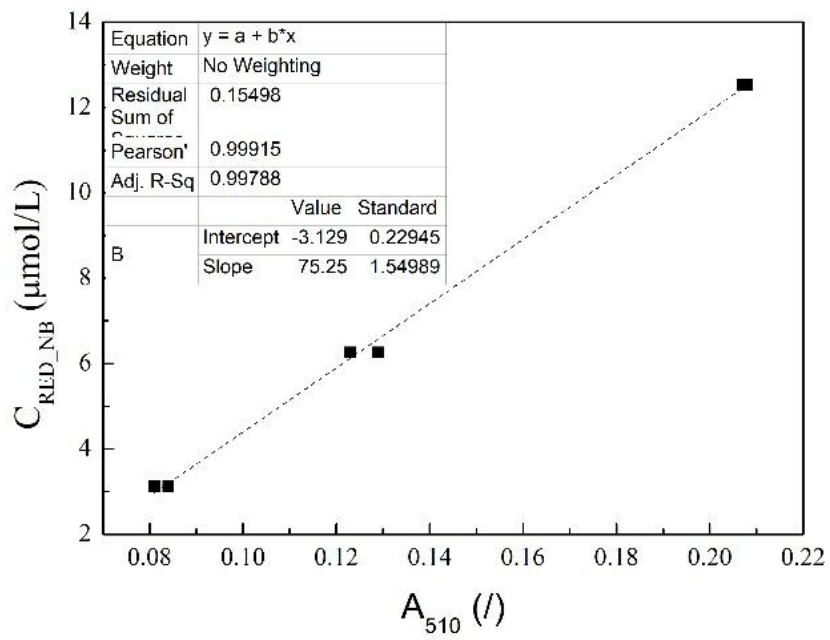
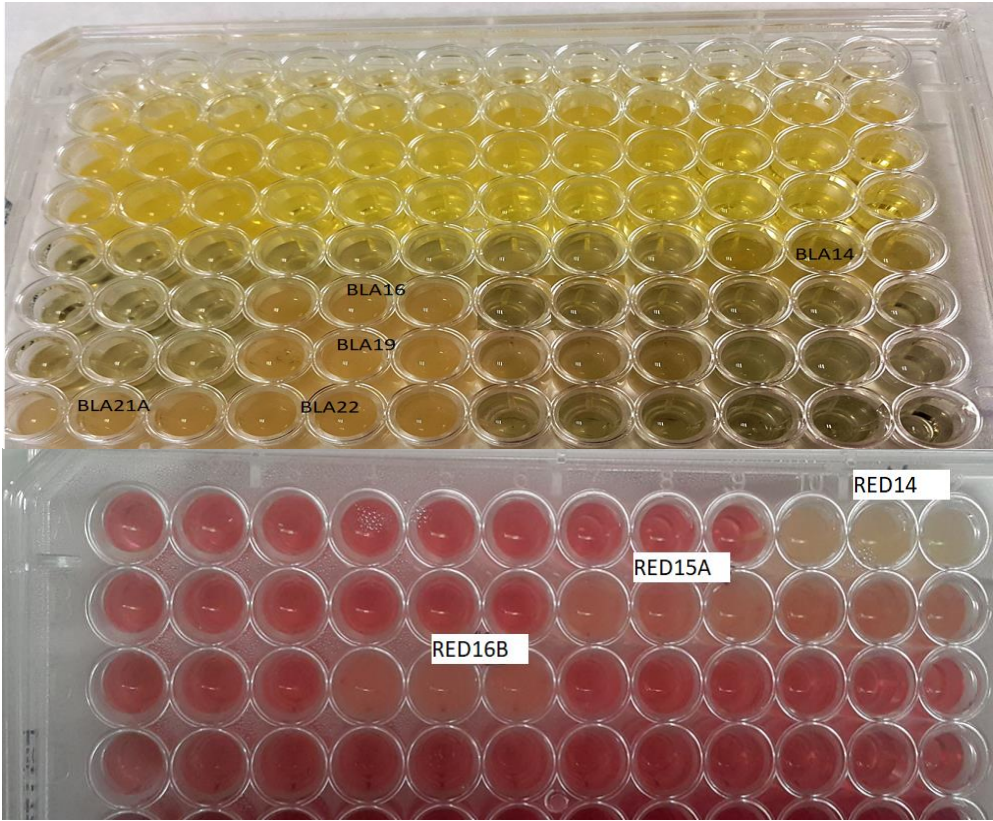
**Supplementary Table A.6.** Results of the time-dependent activity of the most active red, blue and whitener dye degraders for the dye they used during isolation at 25 °C. Degradation is presented in percentages of C/C<sub>0</sub> values ± relative standard errors of triplicates. WH5 and WH8 after 165 h were 10<sup>9</sup> and 6×10<sup>10</sup> CFU/mL, respectively.

Dye	Isolate	C/C <sub>0</sub> λ <sub>max</sub> (%)±RSE (%)				OD <sub>600</sub> (/)±RSE (%)				
		24 h	48 h	72 h	165 h	0 h	24 h	48 h	72 h	165 h
BLU	<b>BLU19</b>	40±3	19±1	13±2	/	0.01±0	0.6±1	0.9±2	0.9±1	/
	<b>BLU23</b>	33±2	17±1	12±2	/	0.01±0	0.6±1	0.9±3	0.8±3	/
RED	<b>RED14</b>	6±3	-7±2	-15±1	/	0.01±1	0.1±1	1.3±4	1±0.5	/
	<b>RED14A</b>	103±0	2.8±3	-14±3	/	0.02±2	0.1±6	1.1±2	1±8.5	/
	<b>RED15A</b>	60±3	-16±0	-16±1	/	0.01±1	0.6±6	1.1±1	1±8	/
	<b>RED16B</b>	65±4	35±2	68±6	/	0.01±1	0.1±7	1.6±5	1.8±5	/
WH	<b>WH8</b>	98±3	92	85±2	75±3	0.00±1	0.03±2	0.04±10	0.06±4	0.3±1
	<b>WH5</b>	96±2	95.5	95±2	84±7	0.00±0	0.00±2	0.00±2	0.01±2	0.01±3

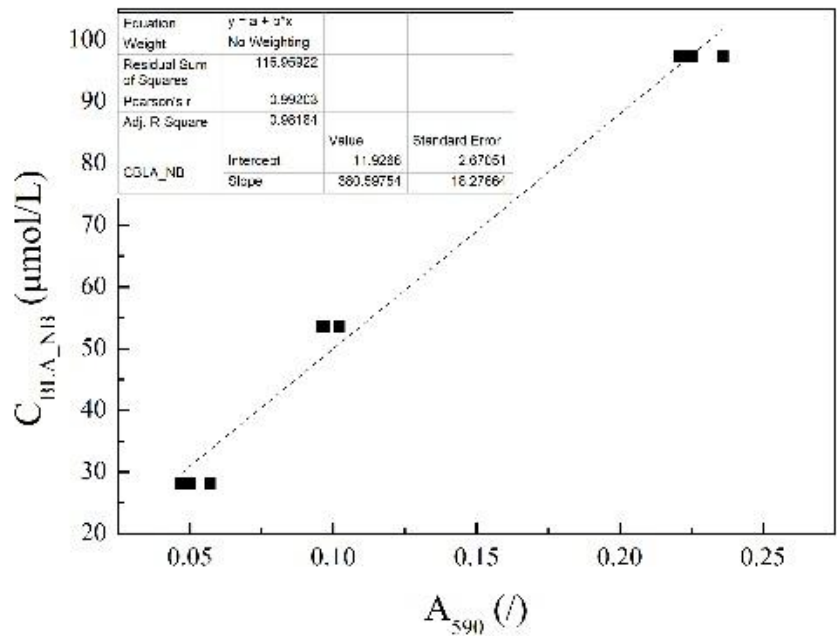
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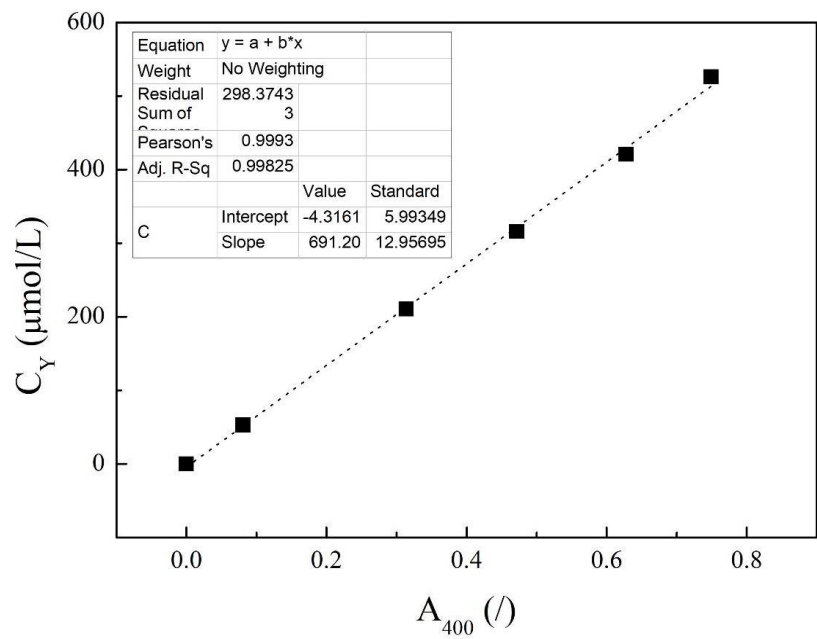
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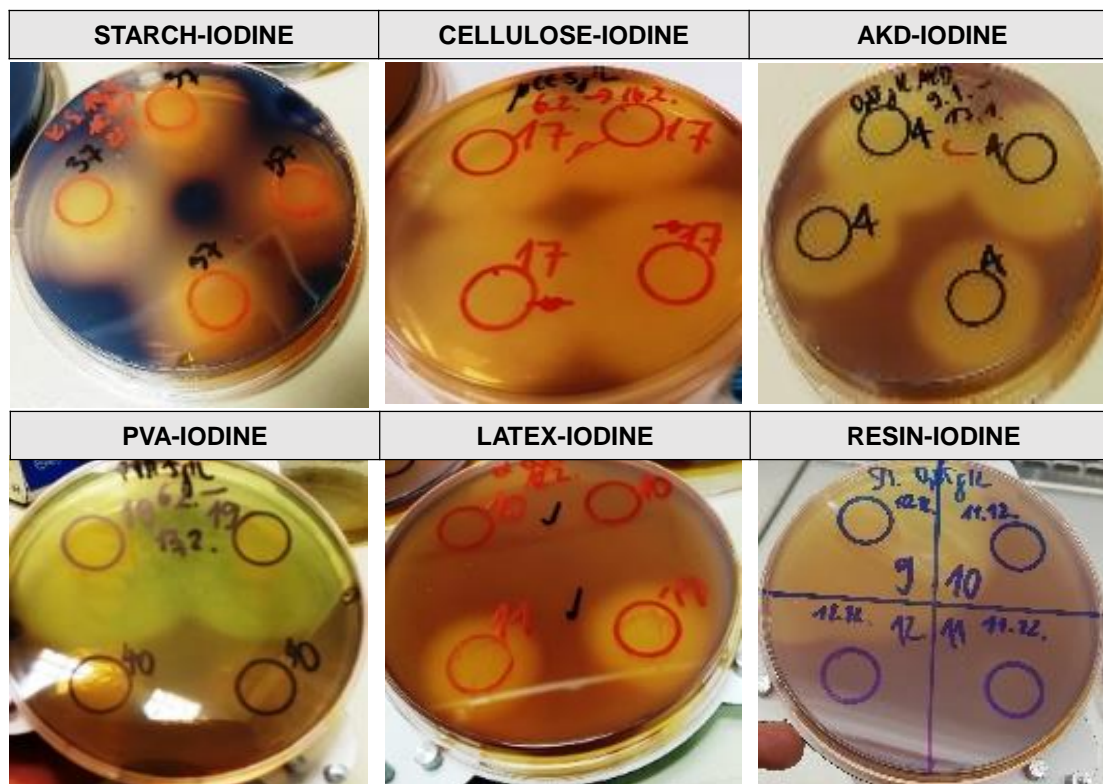
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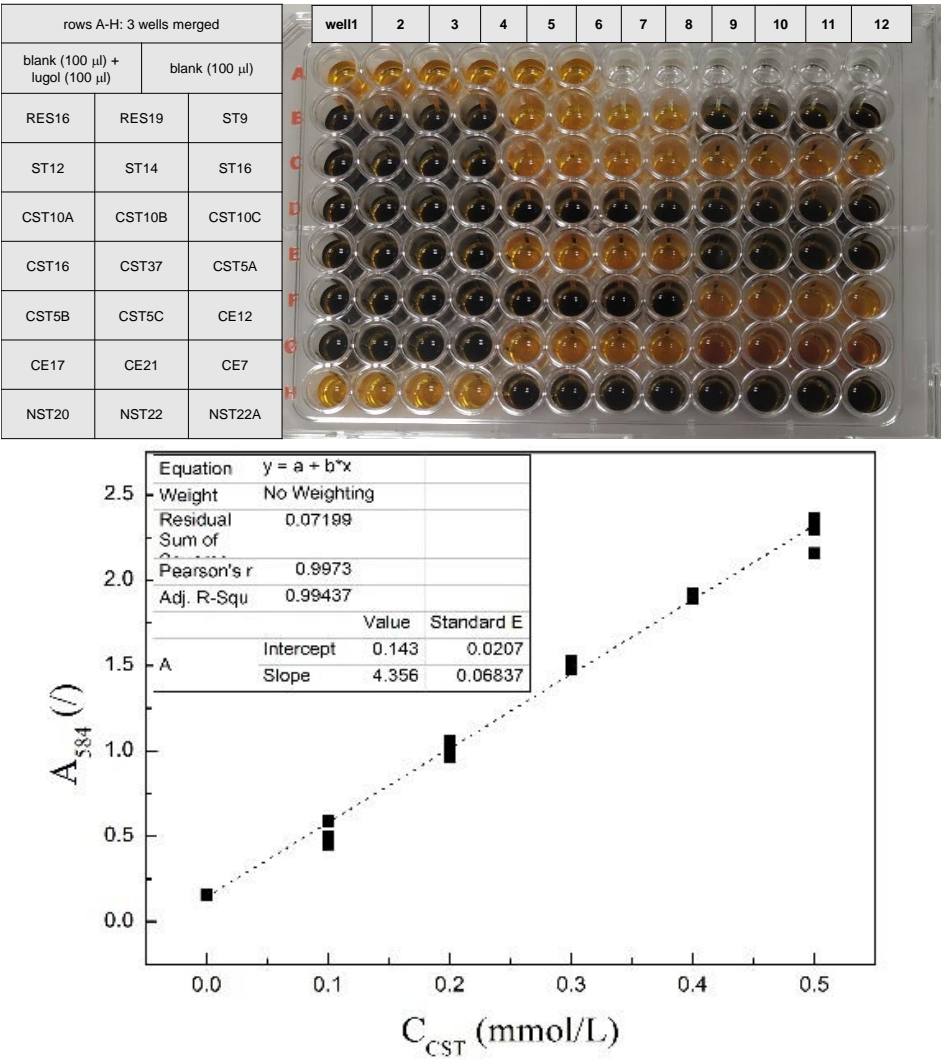
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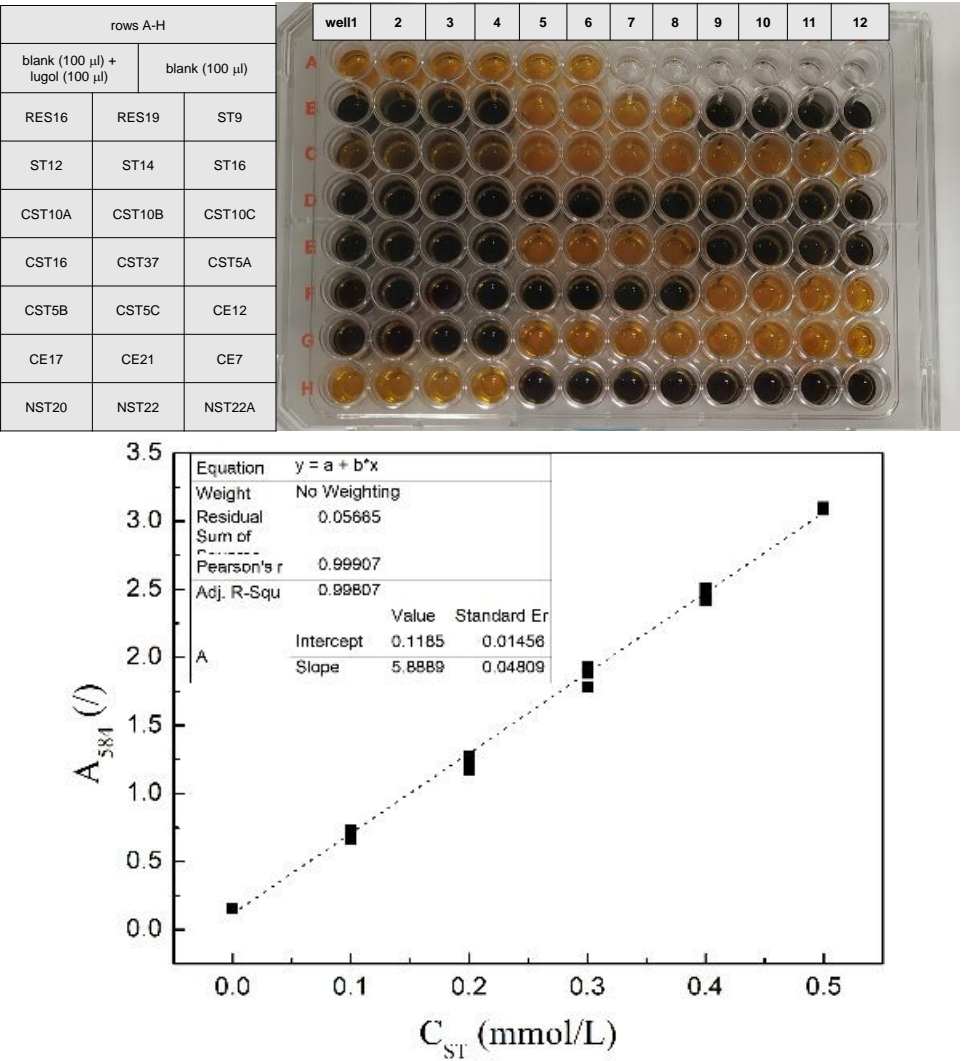
**Supplementary Figure A.1.** Biodegradation assays: (A) above: incubation microtiter plate with 27 BLU isolates in NB with 6 mg/L blue dye, in triplicates, after 18 h at 25°C, below: calibration curve with  $R^2=0.995$ ; (B) above: incubation microtiter plate with 12 RED isolates in NB with 15 mg/L red dye, in triplicates, after 48 h at 25°C, below: calibration curve with  $R^2=0.999$ ; (C) above: microtiter plate rows E-H: 14 BLA isolates in NB with 57 mg/L black dye, in triplicates, after 66 h at 25°C, below: calibration curve for black dye in NB with  $R^2=0.993$ ; (D) calibration curve of yellow dye with  $R^2=0.999$ ; (E) images of M9 agar CST, CE, AKD, PVA, LX and RES after 7-13 days incubation at 25°C without corrections. Above from left to right: Starch-iodine complex formation and distinct discoloring around colonies of CST37, cellulose-iodine complex and distinct discoloring around CE17, AKD-iodine complex and distinct discoloring around AKD4; below from left to right: PVA-iodine complex and distinct discoloring around PVA19 compared to the inactivity of PVA10, LX-iodine complex and distinct discoloration around LX11 compared to the inactivity of LX10 and RES-iodine complex formation and distinct discoloration around RES9 compared to the inactivity of RES10, RES11, RES12.



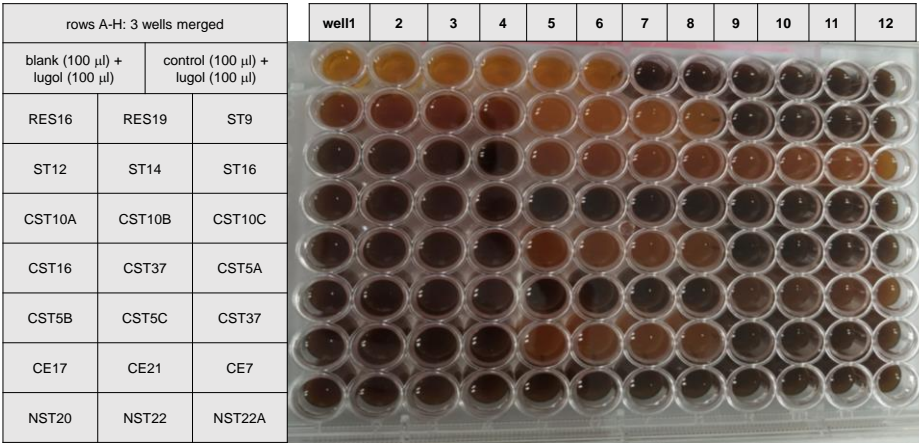
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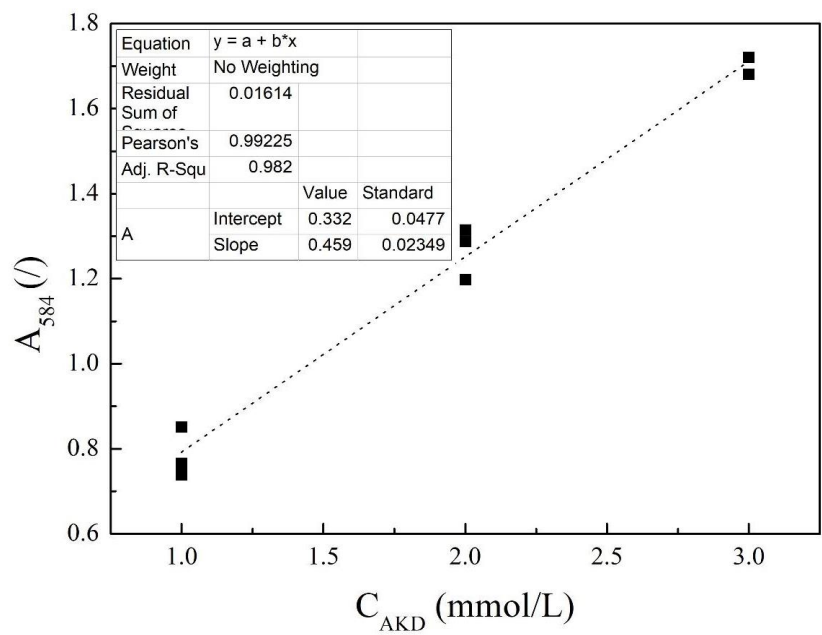


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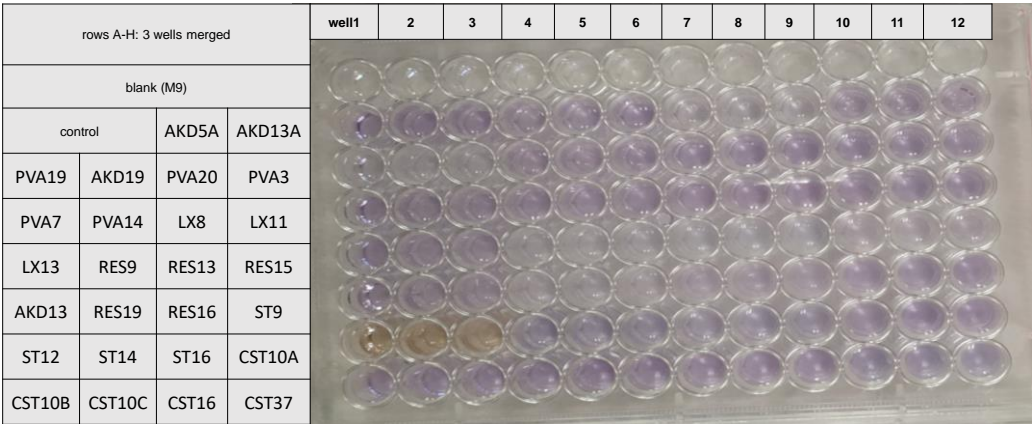
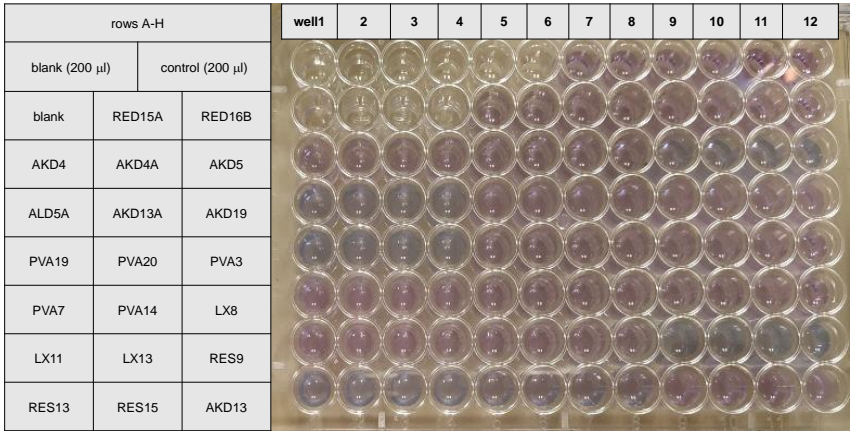


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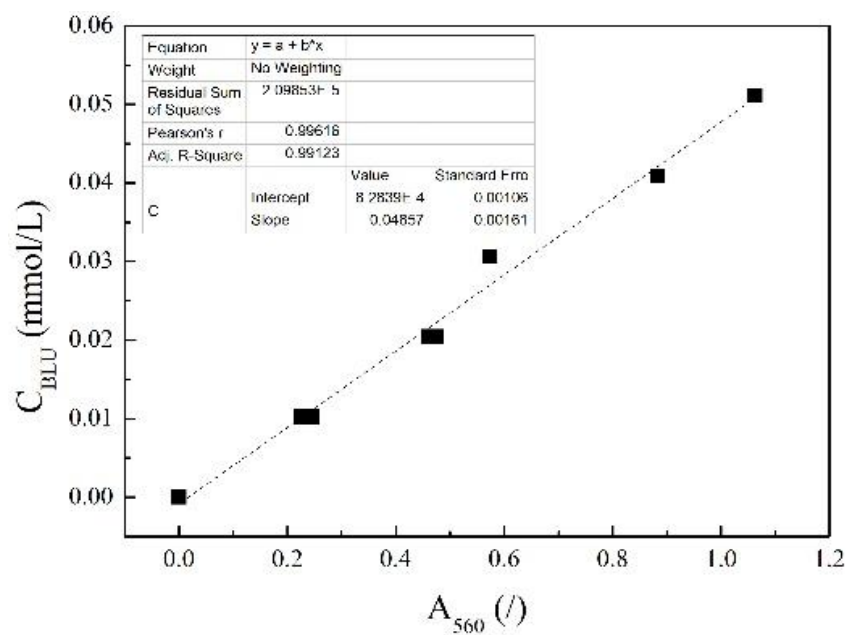




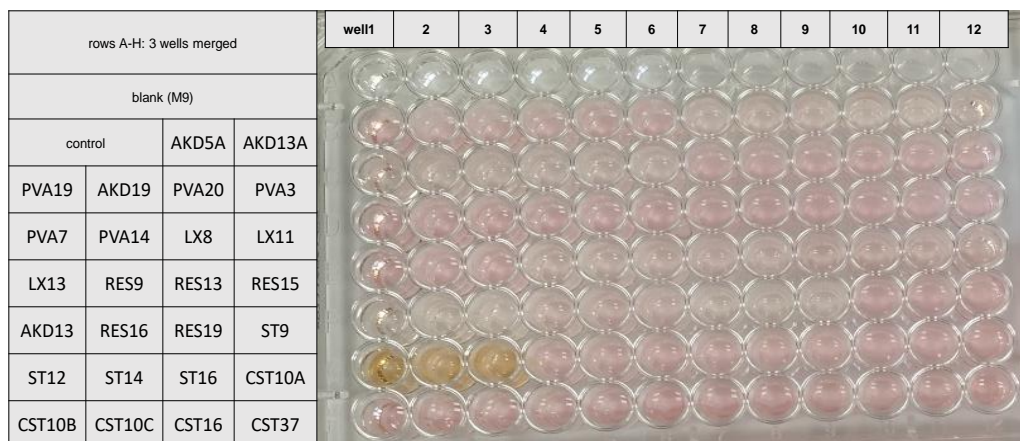
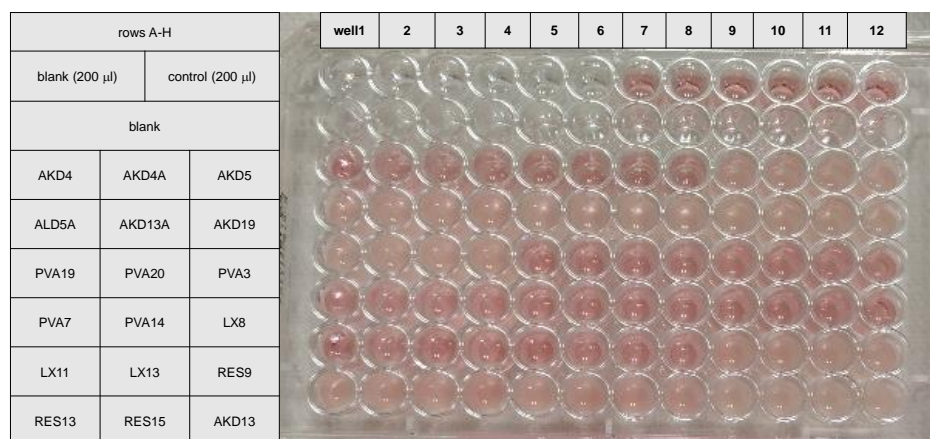
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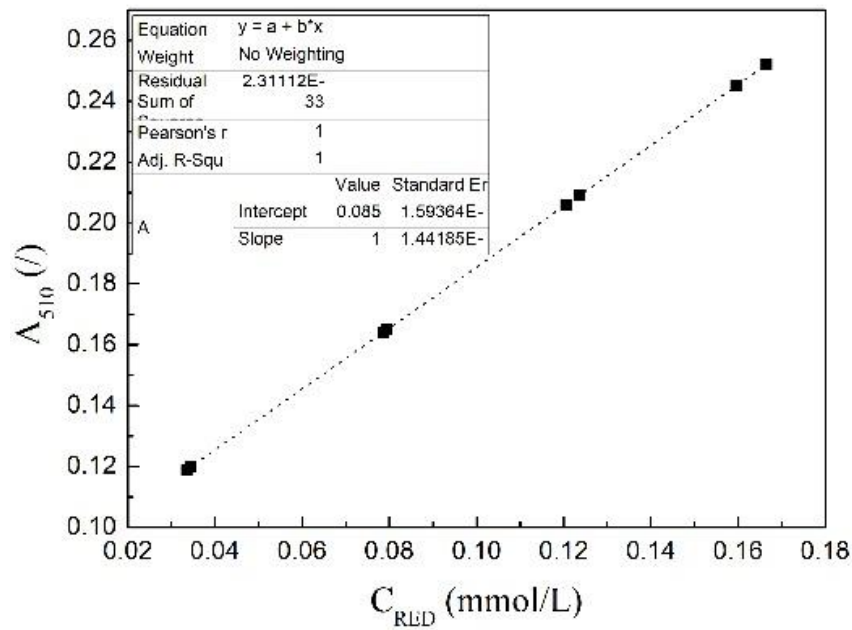




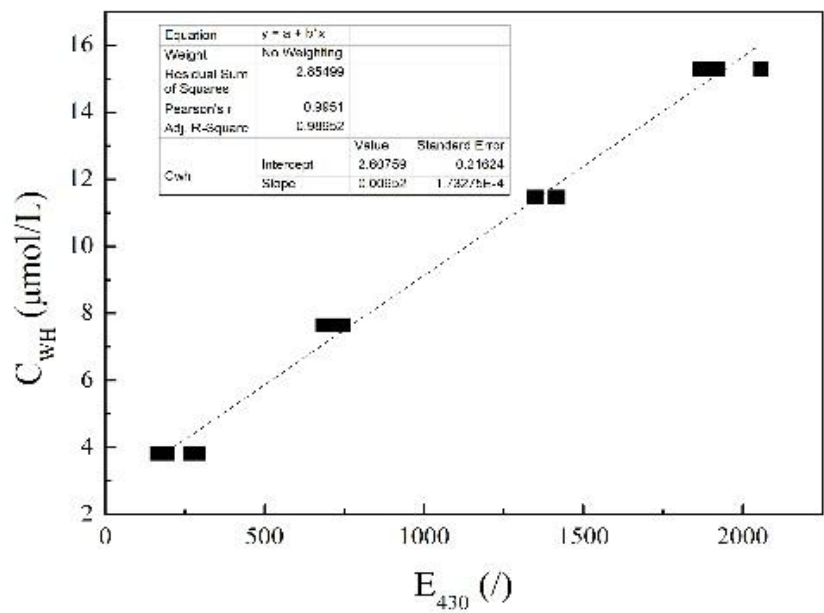


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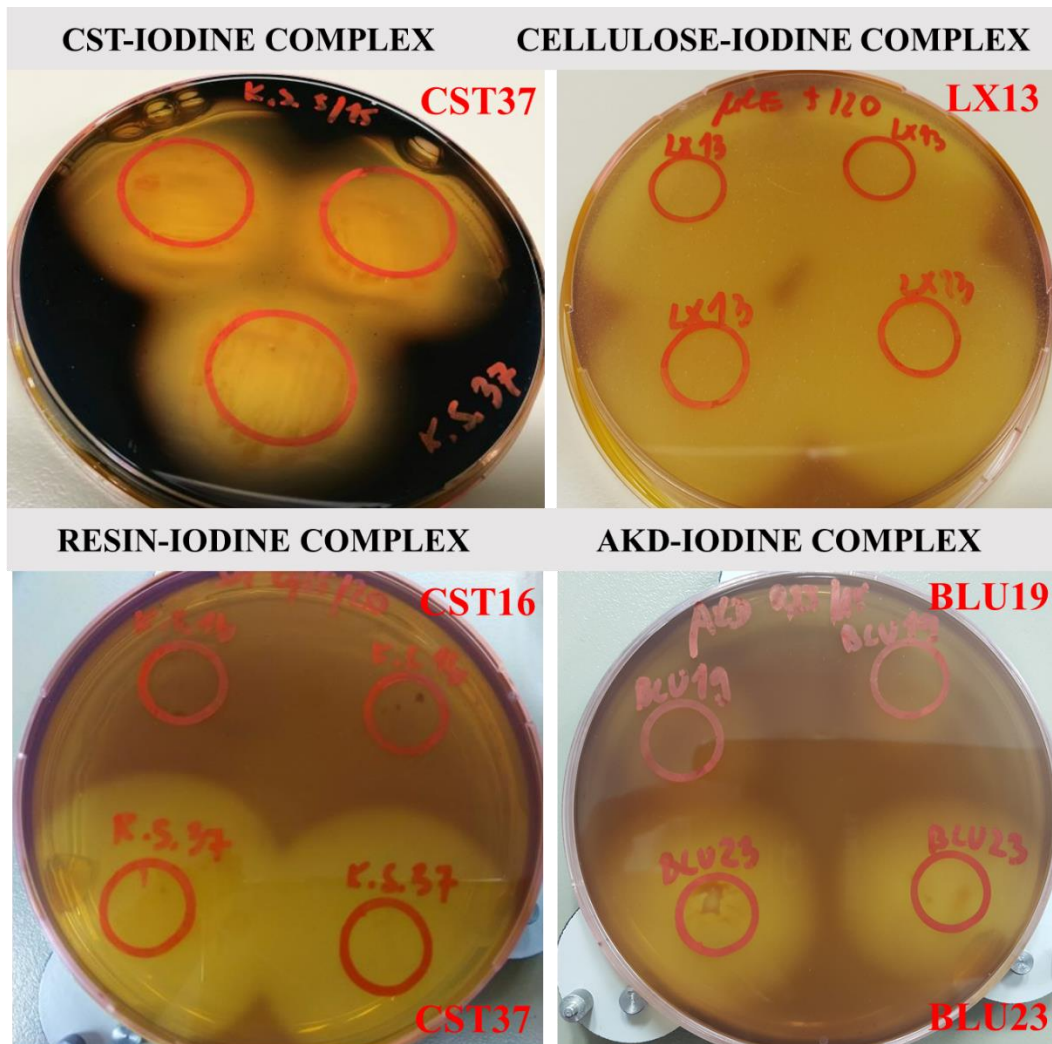




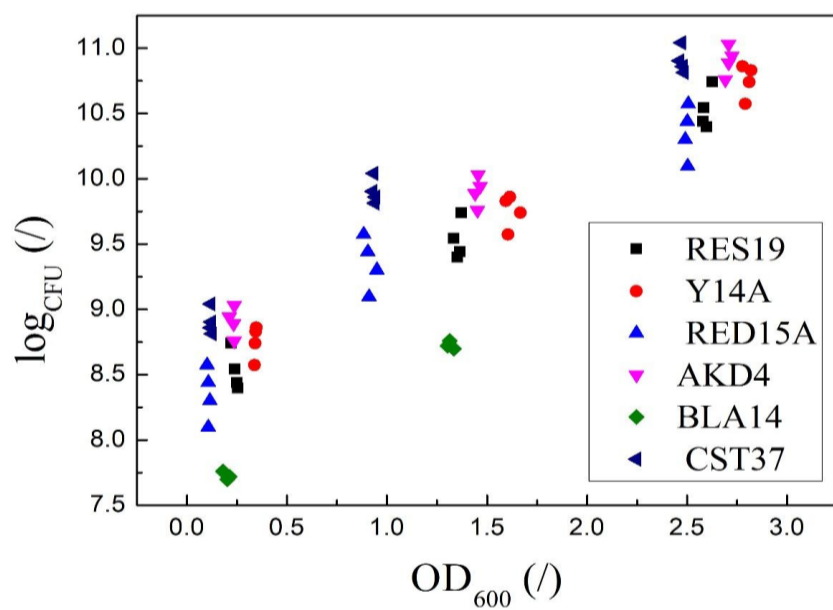
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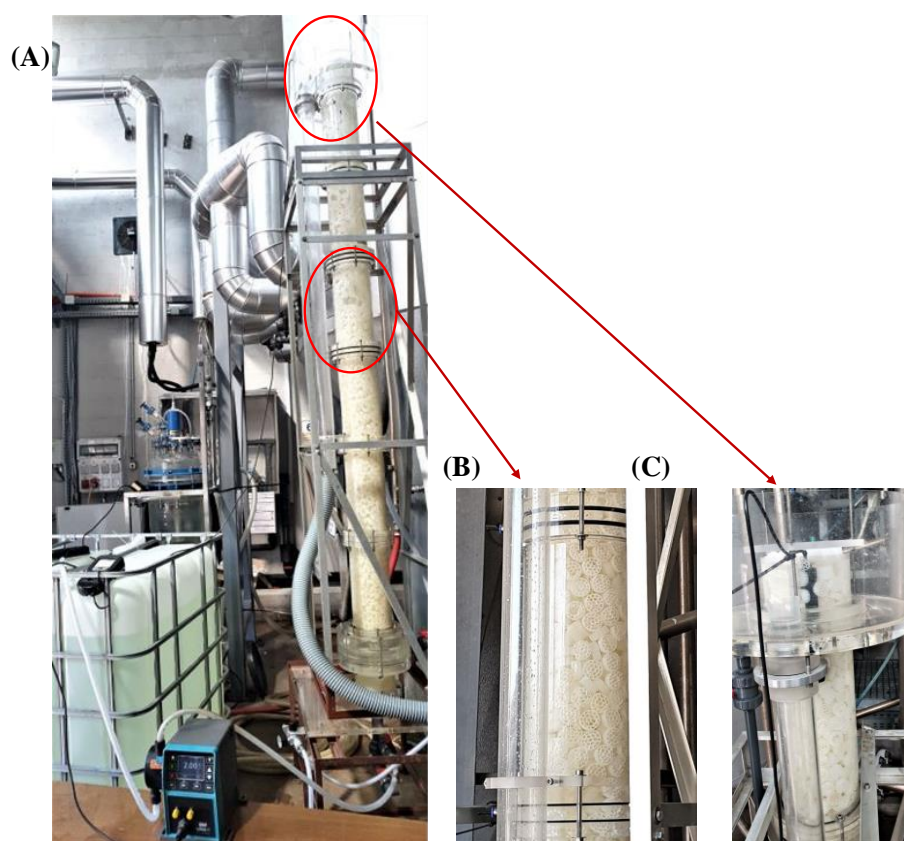
(G)



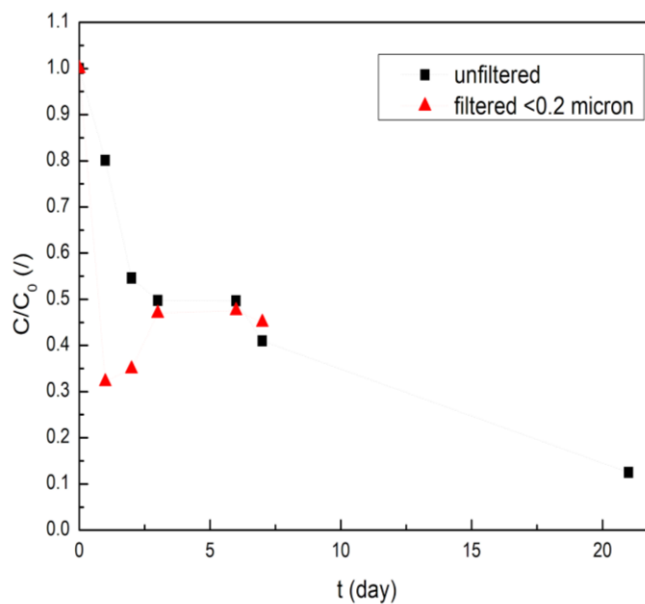
**Supplementary Figure A.2.** Tests for reservoir of carbon source usage. (A) M9 0.5 g/L CST: above: microtiter plate after addition of Lugol, below: calibration curve ( $R^2=0.997$ ); (B) M9 0.5 g/L ST: above: microtiter plate after addition of Lugol, below: calibration curve ( $R^2=0.999$ ); (C) M9 4 g/L AKD: above: microtiter plate after addition of Lugol, below: calibration curve ( $R^2=0.999$ ); (D) BLU in M9<sub>Glc</sub>: above: microtiter plate after 4 days at 25°C, middle: supernatants, below: calibration curve ( $R^2=0.996$ ); (E) RED in M9<sub>Glc</sub>: above: microtiter plate after 4 days at 25°C, middle: supernatants, below: calibration curve ( $R^2=0.999$ ); (F) WH in M9<sub>Glc</sub> calibration curve ( $R^2=0.996$ ); (G) above from left to right: Cationic starch-iodine complex formation after addition of Lugol and distinct discoloration around CST37. Cellulose-iodine complex formation and discoloration due to activities of LX13. Below from left to right: Resin-iodine formation and distinctive discoloration around colonies of CST37 in comparison to inactivity of CST16. AKD-iodine complex formation and discoloration around colonies of BLU23 in comparison to only slight activity of BLU19.



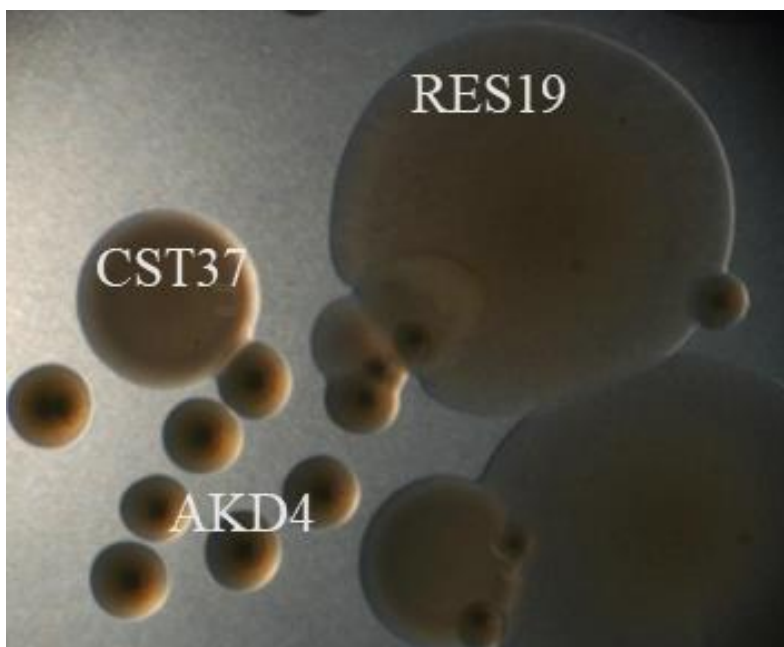
**Supplementary Figure A.3.** Calibration of the growth of the 6 selected bacteria;  $\log(\text{CFU})$  vs.  $\text{OD}_{600}$ .



(D)



(E)

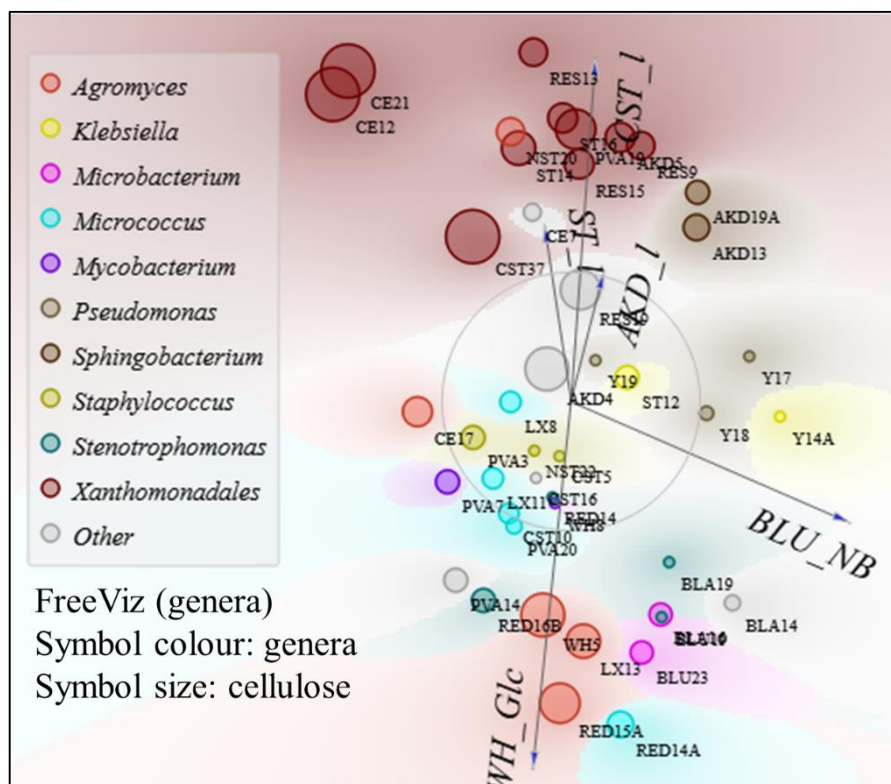


**Supplementary Figure A.4.** Pilot test (for proof of concept only): (A) Photograph of the set-up: a 33 L column with parallel flow of whitewater and air entering at the bottom of the column; (B) part of the column filled with plastic carriers "Kaldens"; (C) upper part of the column with inserted oxygen electrode; (D)  $C/C_0$  from COD measurements of influent and effluent whitewater as a function of time; whitewater samples unfiltered and filtered through 0.2  $\mu\text{m}$  membrane, (E) A representative image of

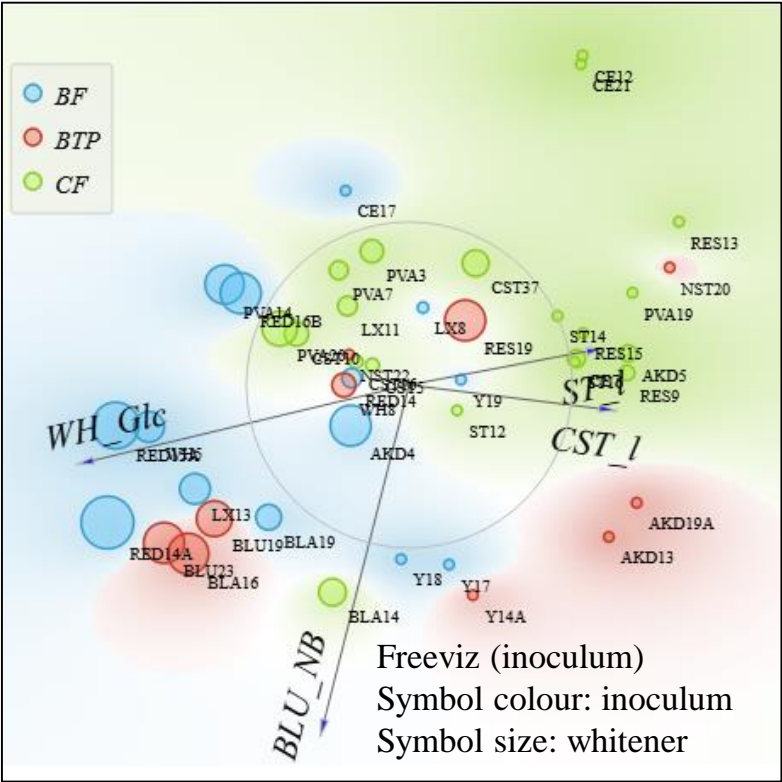


the colonies obtained with the microscope Motic SMZ-168- BL in a parfocal 6.7:1 zoom. The CFU count was as follows: 3×RES19, 2×CST37 and 13×AKD4.

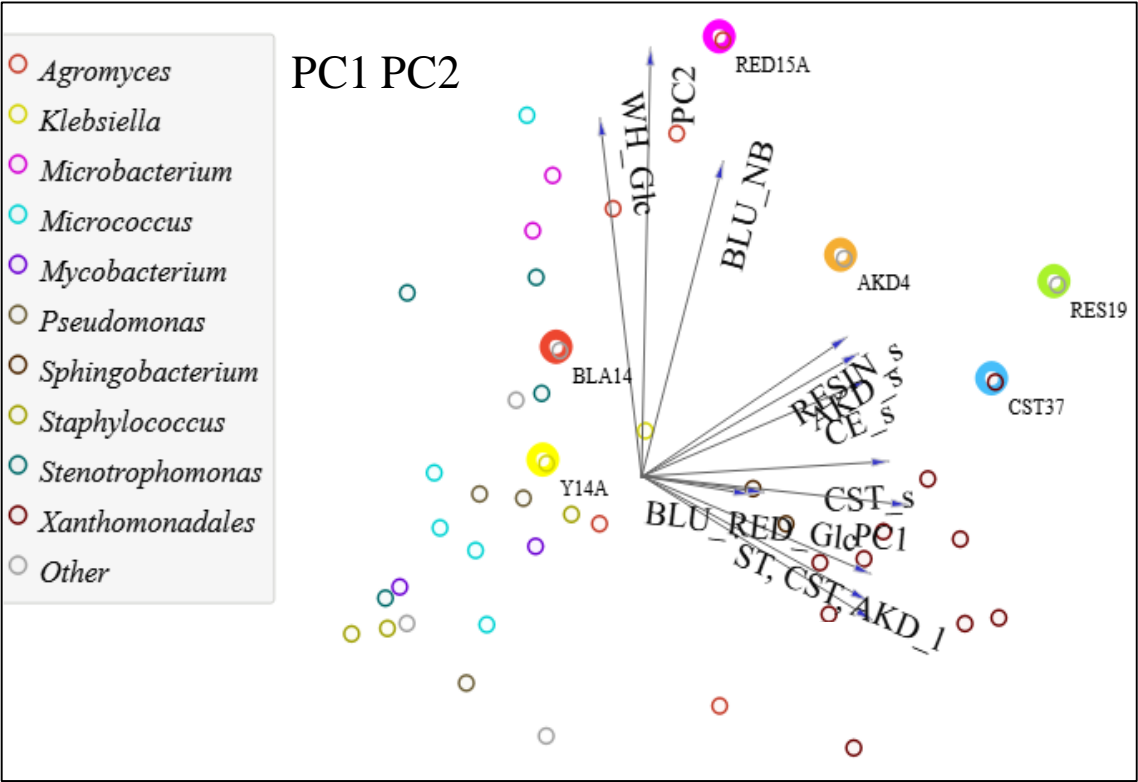
(A)



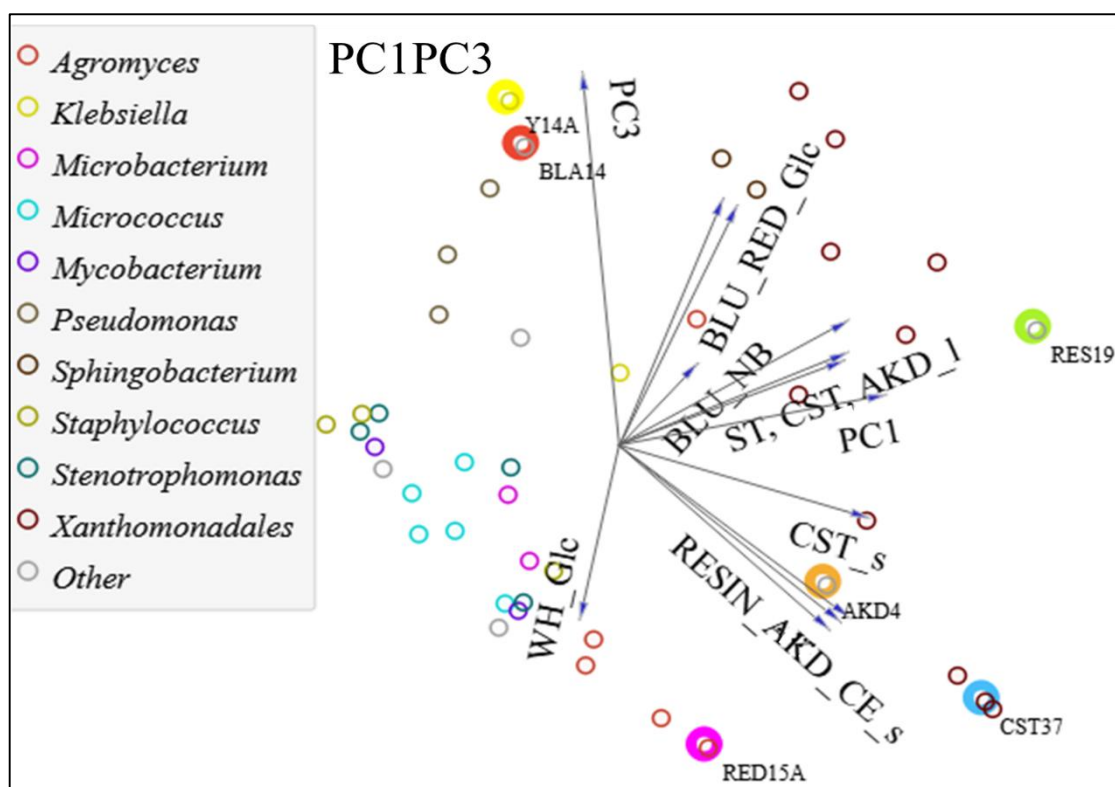
(B)



(C)



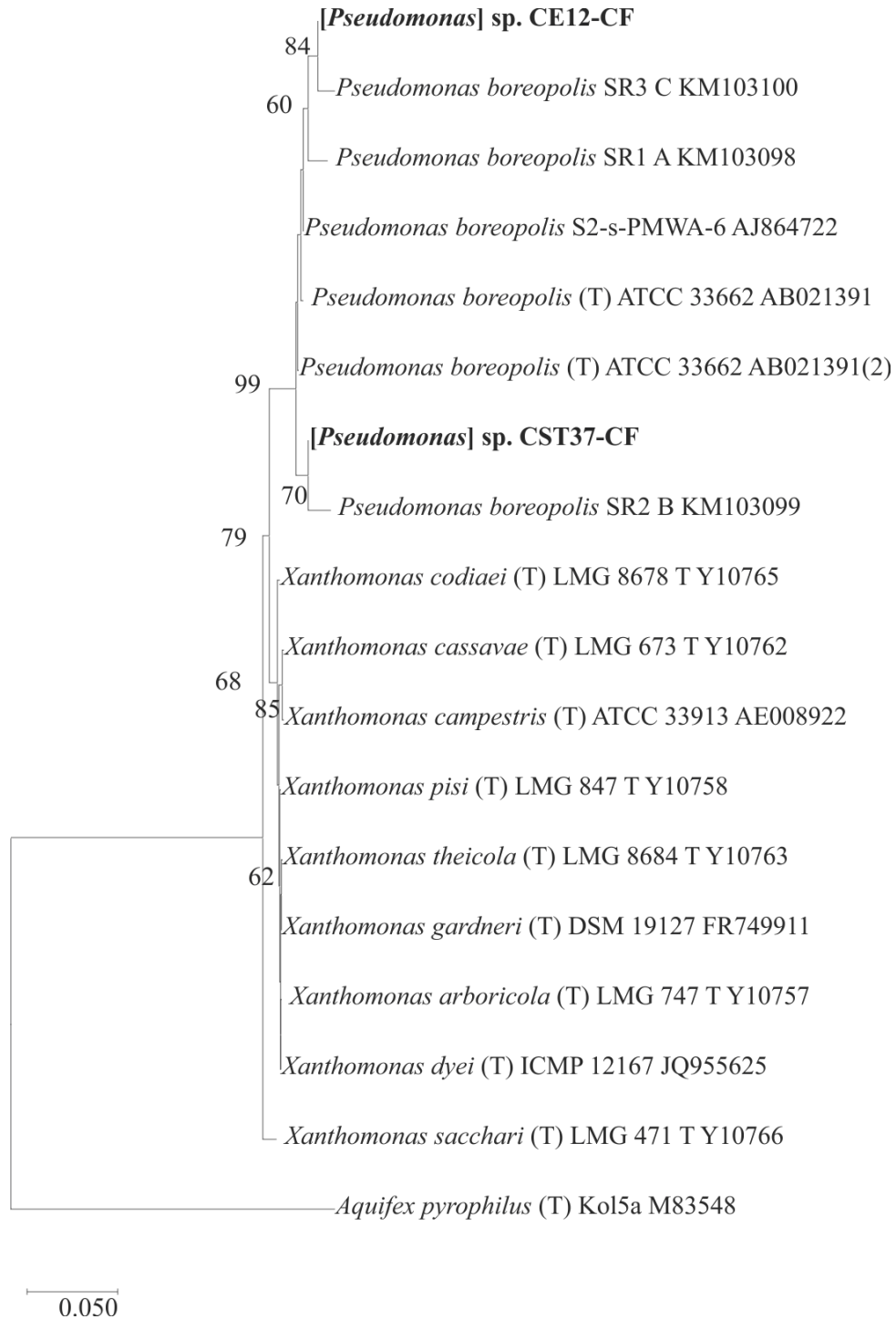
(D)



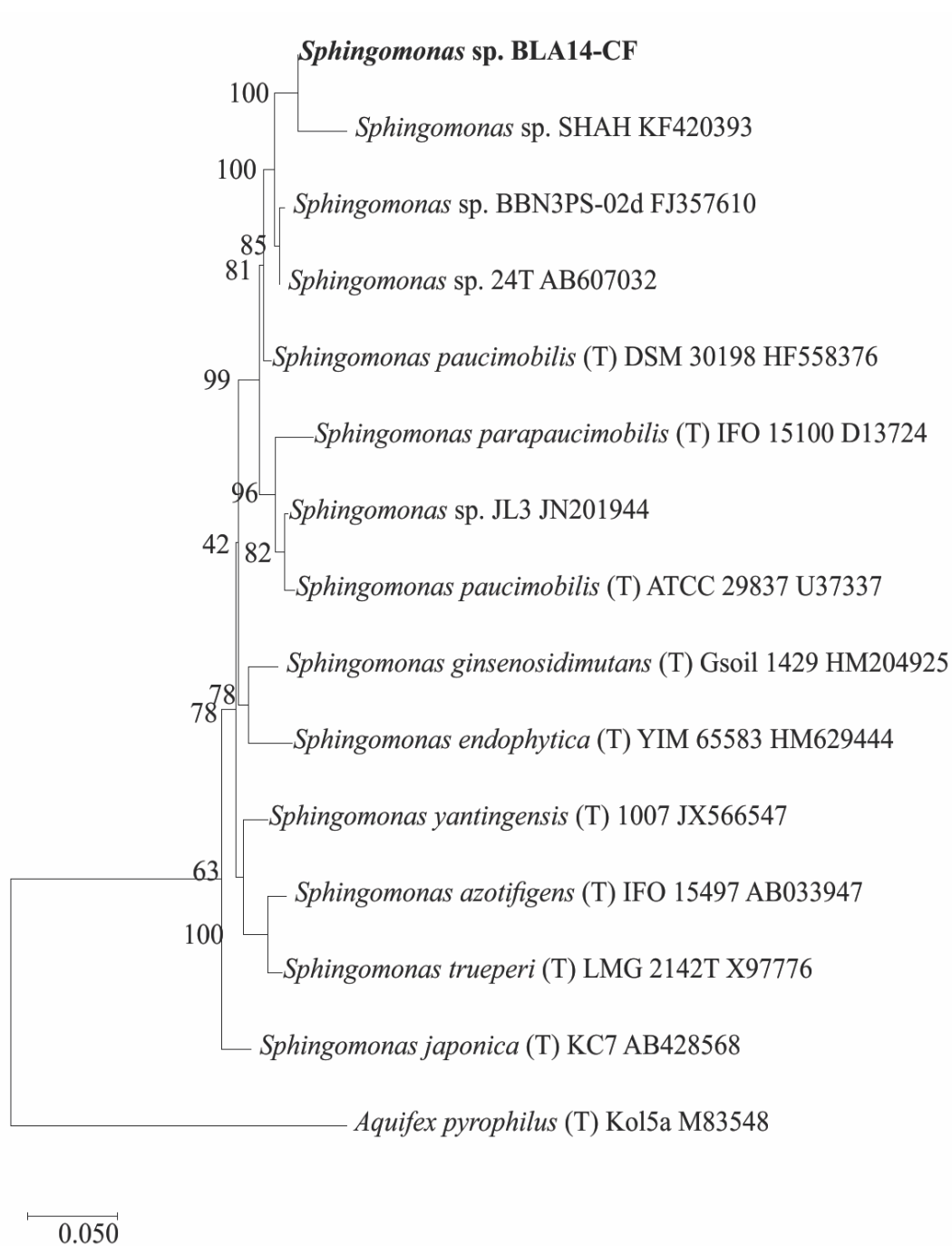
**Supplementary Figure A.5.** FreeViz and PCA of tests for the repertoire of carbon sources of 44 isolates in liquid and solid media. FreeViz projection with (A) clusters of genera and symbol size for cellulose and (B) clusters of inoculum and symbol size for whitener degradation, PCA with clusters for genera and marked 6 selected strains, RED15A (circled in pink), BLA14 (blue), Y14A (yellow), RES19 (green), AKD4 (brown) and CST37 (red): (C) PC1PC2 with 67% and (D) PC1PC3 with 65% explained variances.



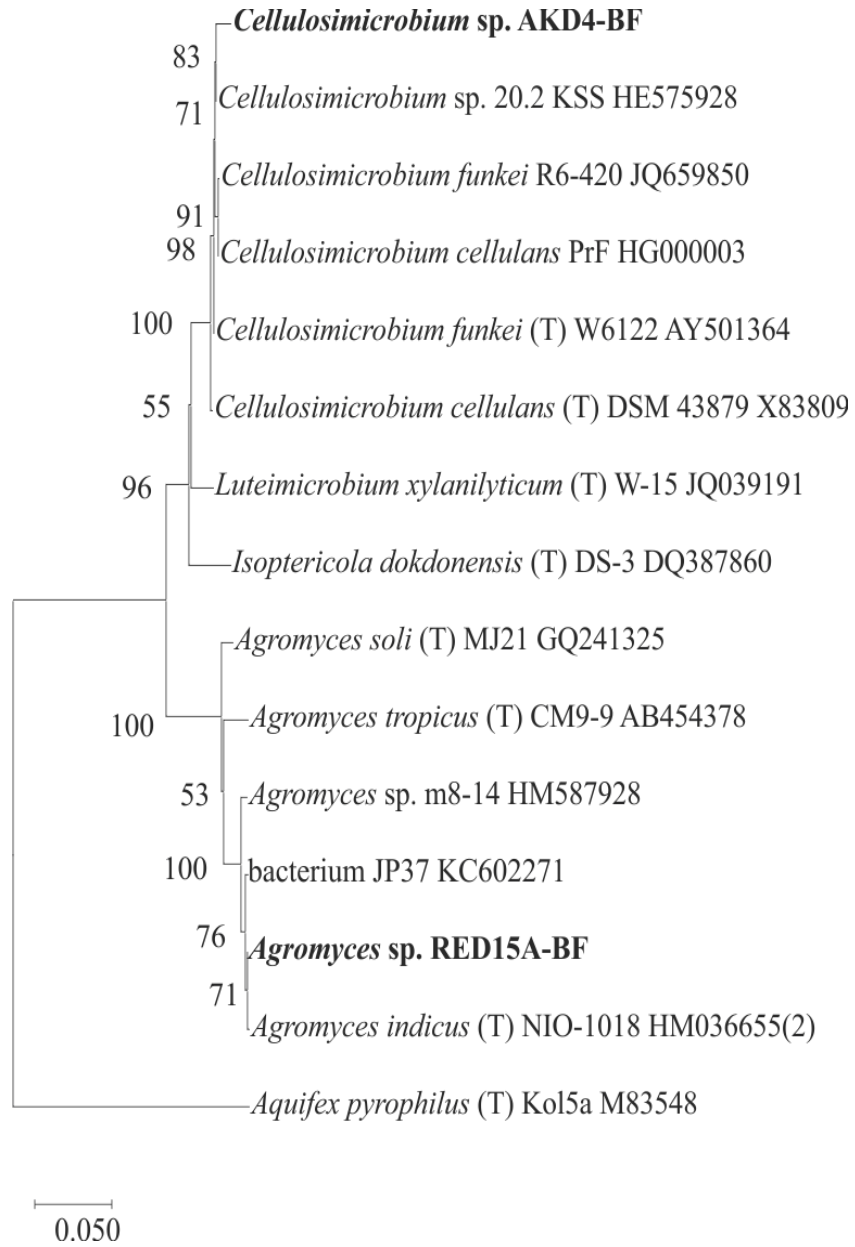
(A)



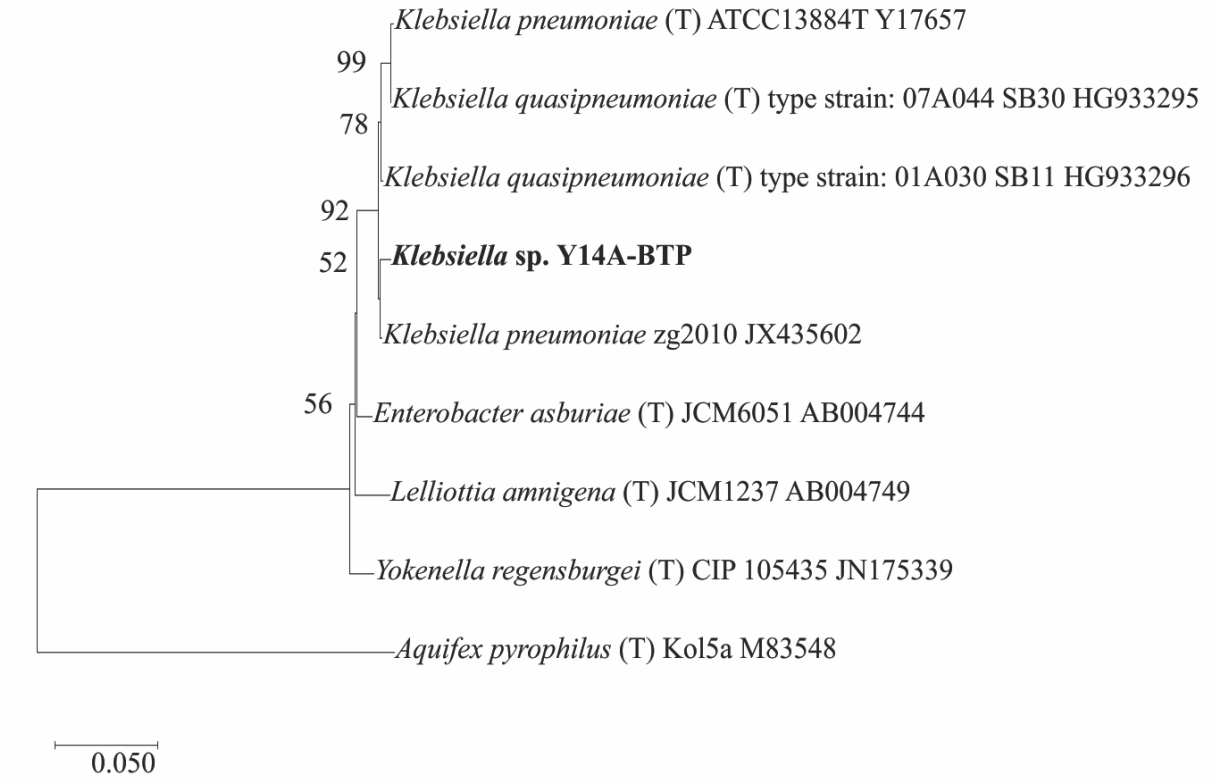
(B)



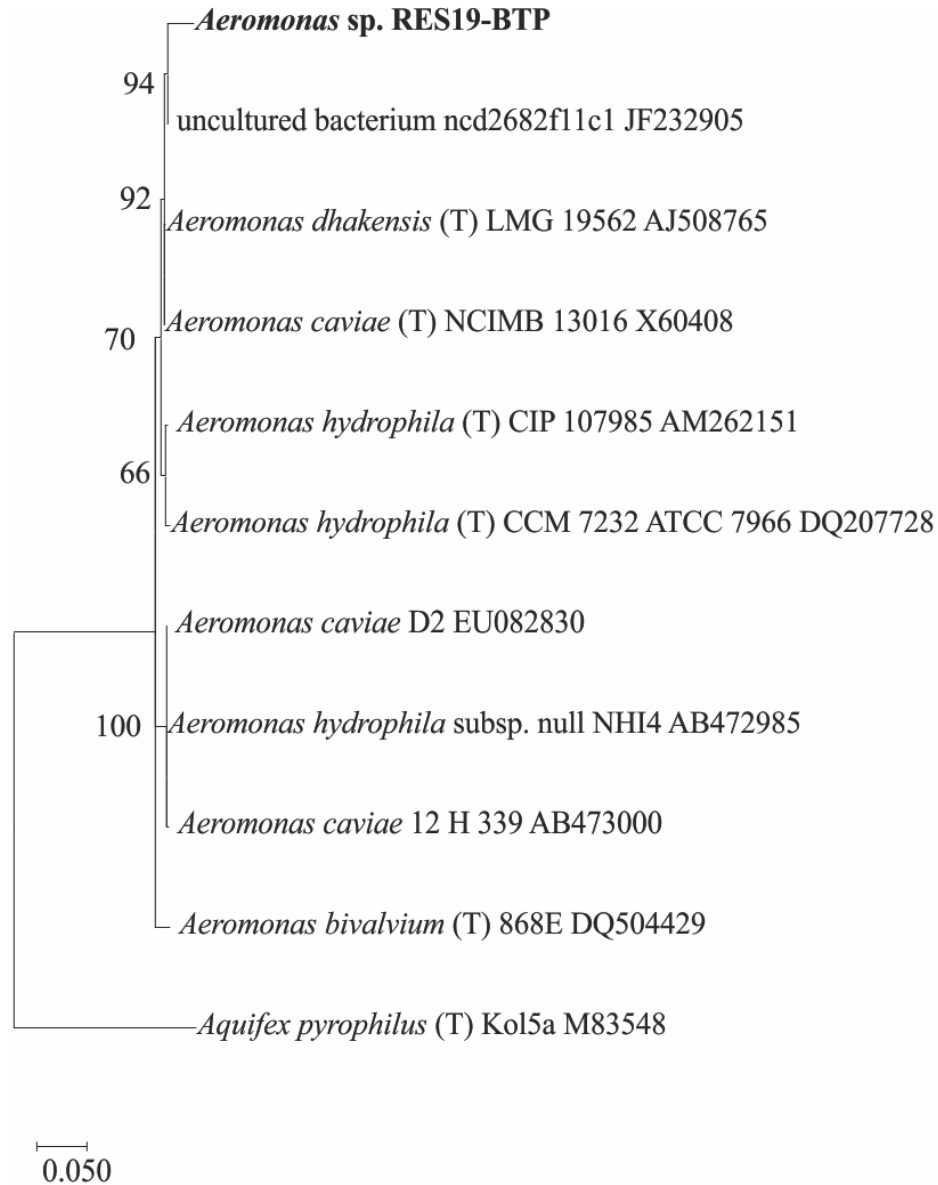
(C)



(D)



(E)



**Supplementary Figure A.6.** Detailed phylogenetic trees displaying the phylogenetic position of six selected isolates with their closest neighbors are presented. *Aquifex pyrophilus* (T) was used as an outlier. Numbers indicate the percentages of bootstrap support, derived from 1000 re-samplings. The evolutionary history was derived using the Neighbor-Joining method (Saitou and Nei, 1987). (A) Xanthomonadales bacterium strain CST37, (B) *Sphingomonas* sp. strain BLA14-CF, (C) *Cellulosimicrobium* sp. strain AKD4-BF together with *Agromyces* sp. strain RED15A, (D) *Klebsiella* sp. strain Y14A-BTP and (E) *Aeromonas* sp. RES19-BTP.