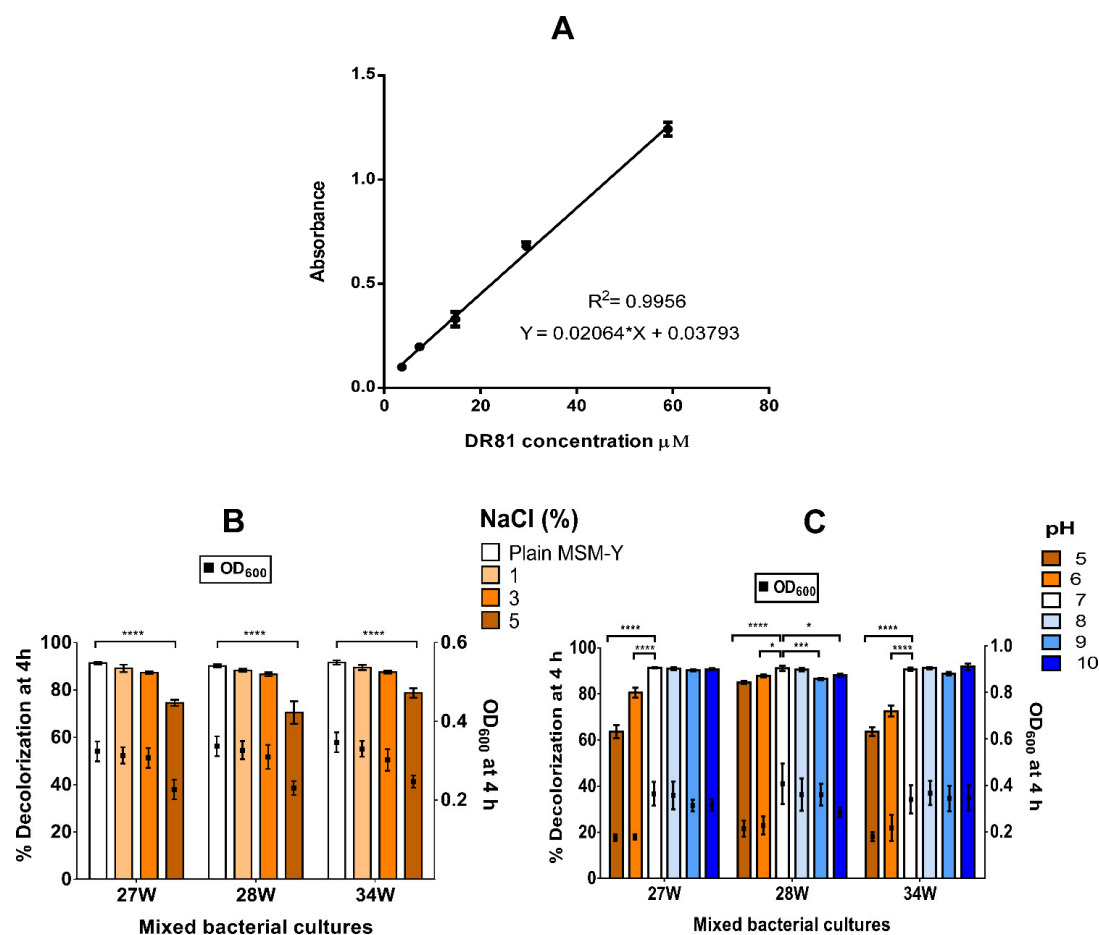
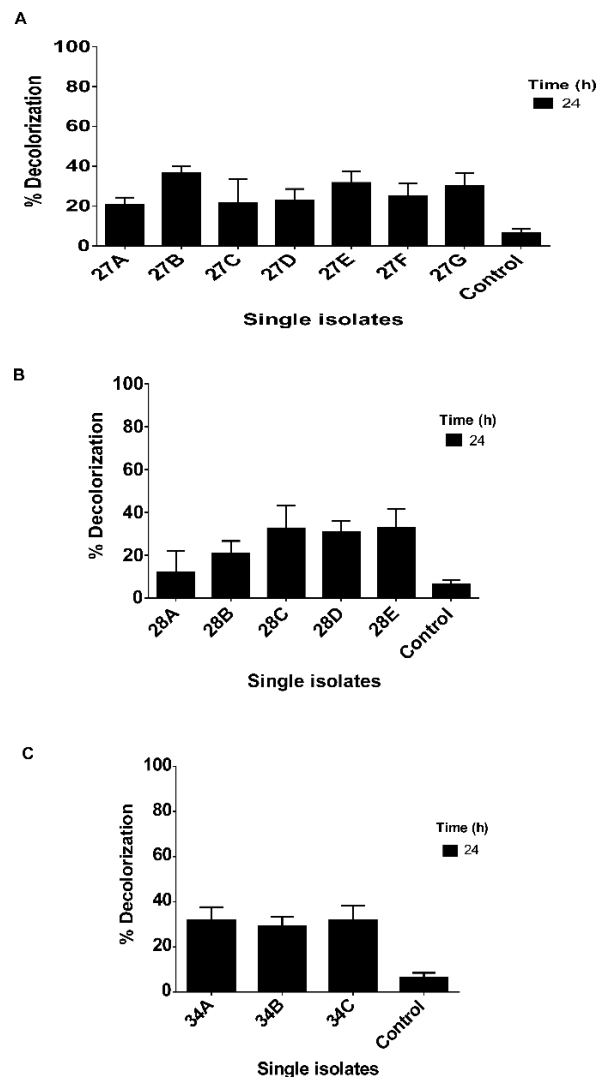


# Supplementary Materials

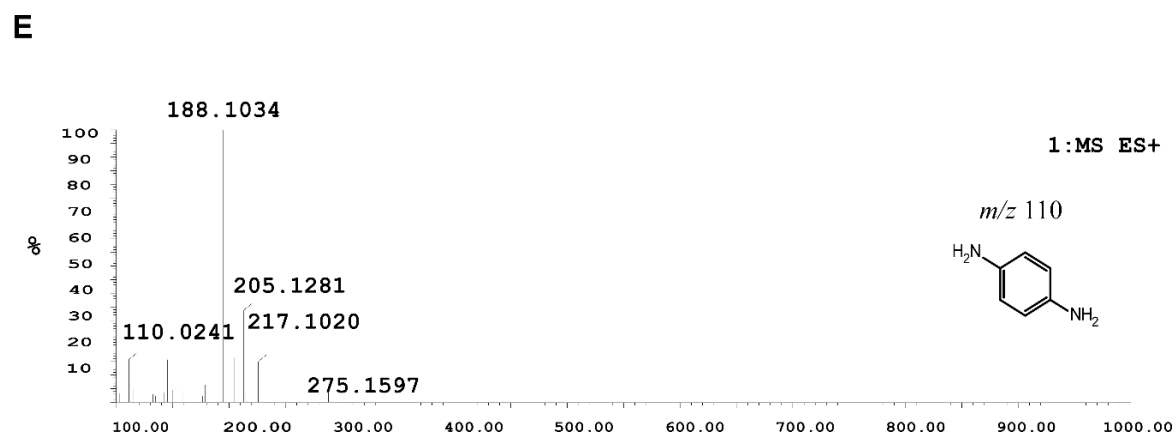
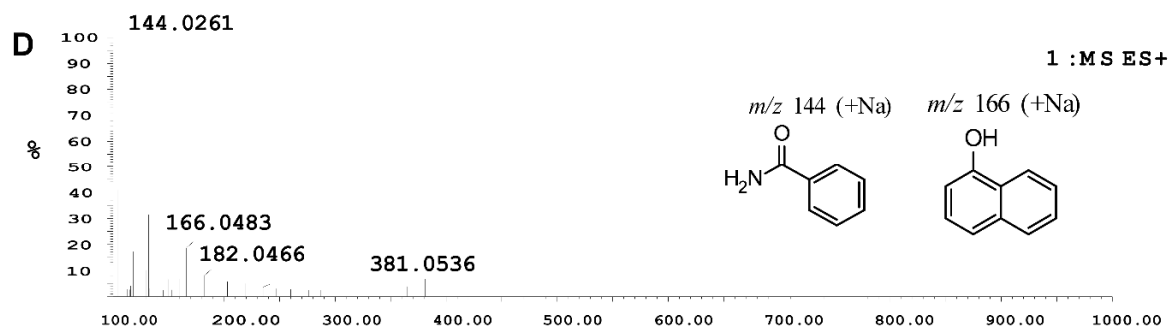
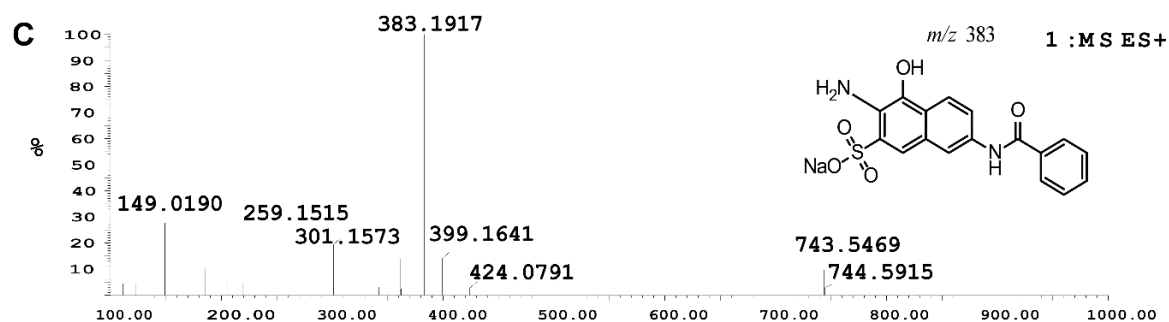
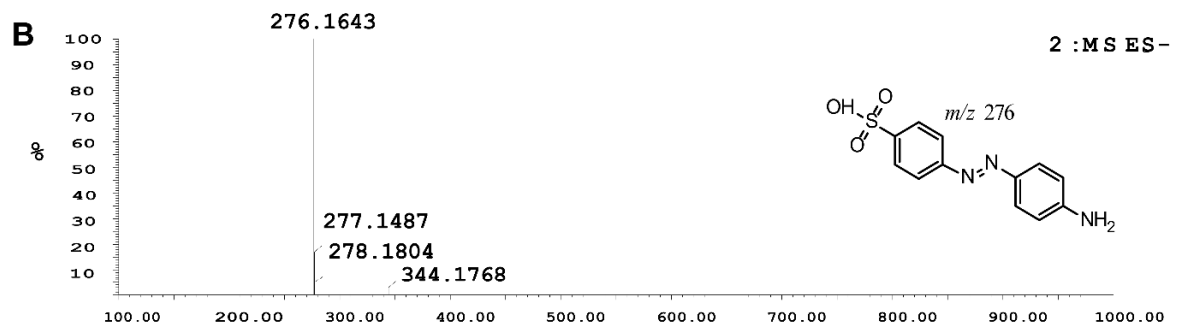
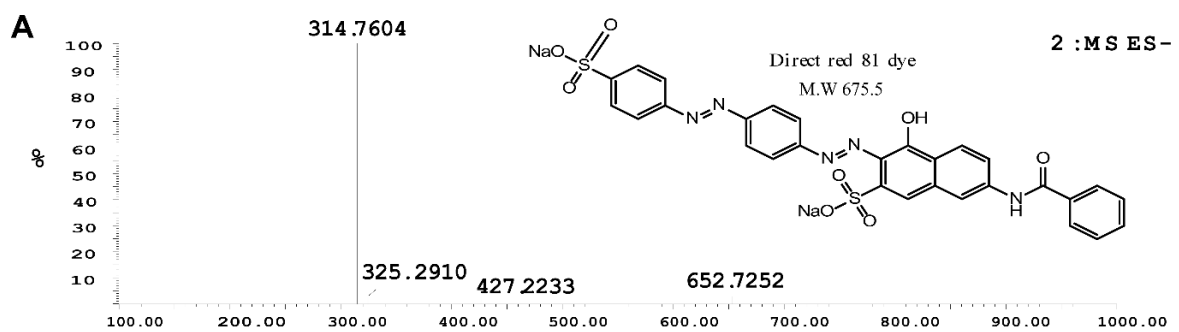
## Supplementary Figures



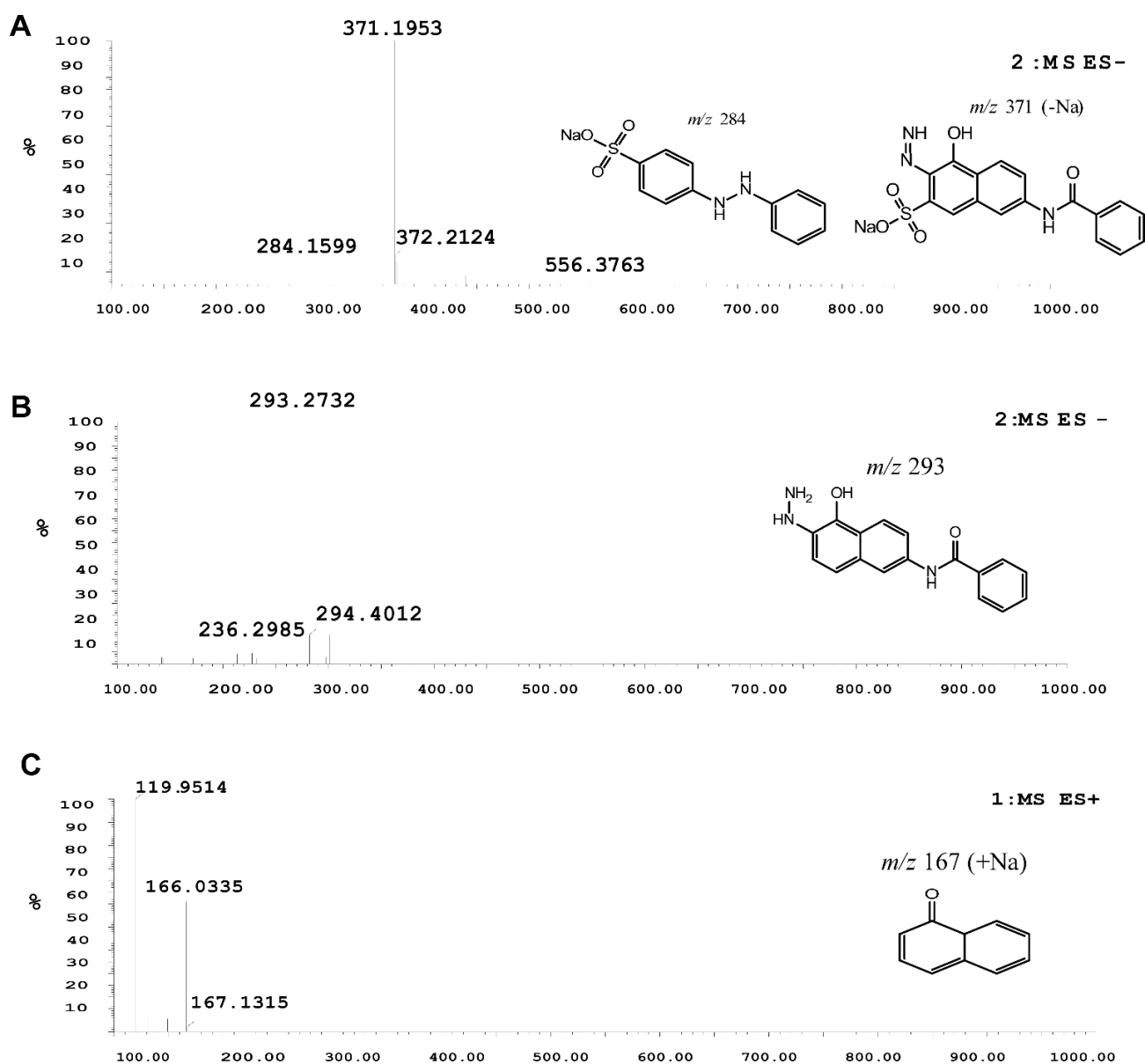
**Figure S1: A panel showing DR81 calibration curve and the effect of both salinity and pH on DR81 decolorization. (A)** Calibration curve for DR81 azo dye. Absorbances were recorded for three separate replicates and results were expressed as mean absorbance  $\pm$  SD, **(B)** Effect of NaCl concentration on DR81 decolorization after 4 h by the selected mixed cultures, **(C)** Effect of pH on DR81 decolorization after 4 h by the selected mixed cultures. Data represent three replicates and analyzed by GraphPad prism using two-way ANOVA with Tukey's multiple comparison test and results were expressed as mean % decolorization  $\pm$  SD. Significance of % decolorization was defined as \*  $p \leq 0.05$ , \*\*  $p \leq 0.01$ , \*\*\*  $p \leq 0.001$ , \*\*\*\*  $p \leq 0.0001$ .



**Figure S2. Decolorization of DR81 by single colonies isolated from the selected mixed bacterial cultures (A) 27W, (B) 28W and (C) 34W after 24 h.** Single isolates showed no decolorization at time intervals 2, 4, 6 and 8 h and no visible complete decolorization was detected at 24 h when compared to their corresponding mixed culture. Data represent at least three replicates and plotted as mean % decolorization  $\pm$  SD using GraphPad prism.



**Figure S3.** Mass spectra, chemical structures, and IUPAC names for the parent dye DR81 and the possible detected metabolites in the resolubilized methanolic extract of culture supernatant from symmetric cleavage by azoreductase extracted after 1 and 2 h incubation, analyzed using HPLC-MS. (A) DR81 parent dye with  $m/z$  314.7 and 652.7 in the ESI-mode (sodium 7-benzamido-4-hydroxy-3-((E)-4-((E)-(4-sulfonatophenyl)diazenyl)phenyl)diazenyl)naphthalene-2-sulfonate). (B) Metabolite with  $m/z$  276 in the ESI- mode (E)-4-((4-aminophenyl)diazenyl)benzenesulfonic acid. (C) Metabolite with  $m/z$  383 in the ESI+ mode (sodium 3-amino-7-benzamido-4-hydroxynaphthalene-2-sulfonate). (D) Metabolites in the ESI+ mode with  $m/z$  166 (naphthalen-1-ol) and metabolite with  $m/z$  144 (benzamide). (E) Metabolite with  $m/z$  110 in the ESI+ mode (benzene-1,4-diamine).



**Figure S4.** Mass spectra, chemical structures, and IUPAC names for the possible detected metabolites in the resolubilized methanolic extract of culture supernatant from asymmetric reductive cleavage extracted after 1 and 2 h incubation, analyzed using HPLC-MS. (A) Metabolites in the ESI- mode with  $m/z$  371 (sodium 7-benzamido-3-diazenyl-4-hydroxynaphthalene-2-sulfonate) and  $m/z$  284 (sodium 4-(2-phenylhydrazinyl)benzenesulfonate). (B) Metabolite with  $m/z$  293 in the ESI- mode N-(6-hydrazinyl-5-hydroxynaphthalen-2-yl)benzamide. (C) Metabolite with  $m/z$  167 in the ESI+ mode (naphthalen-1(8aH)-one).

## Supplementary Tables

**Table S1.** Physicochemical parameters of non-sterile wastewater (NSWW) effluent from tanning industrial zone before treatment and after 8 h treatment with selected mixed cultures.

Parameters	Untreated wastewater at 0 time	Treated wastewater after 8 h by selected mixed cultures		
		27W	28W	34W
pH	8.94	8.76	8.68	8.6
TDS <sup>1</sup>	1450	1150	1050	1035
TSS <sup>2</sup>	88	56	50	43
COD <sup>3</sup>	2842	694	595	505
BOD <sup>4</sup>	500	50	45	41
Phosphate <sup>5</sup>	60	16.3	15.9	13.8
Ammonium	10	< 0.01	< 0.01	< 0.01
Nitrate	0.6	0.3	0.25	0.25
Total nitrogen	0.13	0.07	0.05	0.05
Phenols	3.2	1.88	1.5	1.38
Carbonate	nil	nil	nil	nil
Bicarbonate	976	10.67	15	10
Total hardness	740	680	590	501

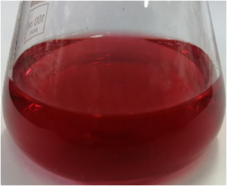
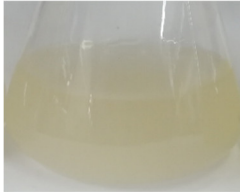
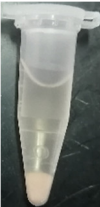
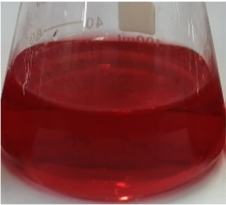
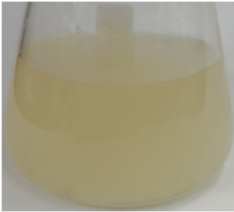

<sup>1</sup> TDS: total dissolved solids, <sup>2</sup>TSS total suspended solids, <sup>3</sup>COD chemical oxygen demand, <sup>4</sup>BOD biological oxygen demand. <sup>5</sup>All parameters were expressed in mg/l, except pH.

**Table S2:** Primers used for PCR and sequencing of azoreductase genes in the selected mixed cultures

Name	Primer Sequence (5-3')	Microorganism	Product size (bp)	Annealing temp (°C)
<i>Alc_F_1</i>	ATGAAACTGCTACACGTAGA	<i>Alcaligenes faecalis</i>	617	49.1
<i>Alc_R_601</i>	CTCTTTTCCAGCAAGGC			
<i>Pch_F_29</i>	ACACGGGCAAGGGTCTG	<i>Pseudochrobactrum saccharolyticum</i>	524	47.7
<i>Pch_R_533</i>	GTTCTCGTTTTTGCCGGATC			
<i>Ach_F_1_8</i>	ATGAAGACCCTCGTCATCC	<i>Achromobacter xylosoxidans</i>	626	51.2
<i>Ach_R_612_8</i>	GCCTGCCGCGAGACT			
<i>Sten_F_1</i> <sup>a</sup>	ATGAAGCTTCTGCAYMTCGA	<i>Stenotrophomonas maltophilia</i>	574	50.2
<i>Sten_R_558</i>	CTTCGACCACTTCGTGC			
<i>Ps_F_61</i> <sup>a</sup>	RCTCATGTTCAGGCC	<i>Pseudomonas monteilii</i>	505	49.1
<i>Ps_R_549</i>	CCAGCAGGATTCCGTTT			
<i>Ps_F_18</i>	CGATTGAGCATCCTGG	<i>Pseudomonas putida</i>	583	49.1
<i>Ps_R_583</i>	AAACAGCTCGTTGGCAAT			
<i>Ps_F_1</i> <sup>a</sup>	ATGAAACTGYTGYACATCGA	<i>Pseudomonas taiwanensis</i>	593	51.2
<i>Ps_R_574</i> <sup>a</sup>	KCGYTGGCAATCTGCGCCTG			
<i>Pr_F_1</i>	ATGAAAAAGATCCTTGTACT	<i>Proteus vulgaris</i>	592	42
<i>Pr_R_573</i> <sup>a</sup>	CAGYATCAATTTCTTTTGT			
<i>Paen_F_22</i>	ACTGCCCATCCTCATGATCA	<i>Paenibacillus taichungensis</i>	518	44.6
<i>Paen_R_520</i>	ACAAAAATGCCTTCAAGCTT			
<i>U4</i>	AGG CCC GGG AAC GTA TTC AC	16S rRNA gene	481	52
<i>U5T</i>	TCA AAT GAA TTG ACG GGG GC			

<sup>a</sup> Degenerate primer

**Table S3.** Decolorization of DR81 azo dye by selected bacterial mixed cultures showing both decolorized suspensions and cell pellets.

Azo dye	Mixed cultures	Before decolorization	After decolorization	
			Suspension	Supernatant and cell pellets
Direct red 81	27W			
	28W			
	34W	