A Sustainable Bioinspired Nano-assembly of Live Marine Bacteria for Mineralization of Phenothiazine Dye

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Supplementary Information

1. Characterization of GO nanosheets

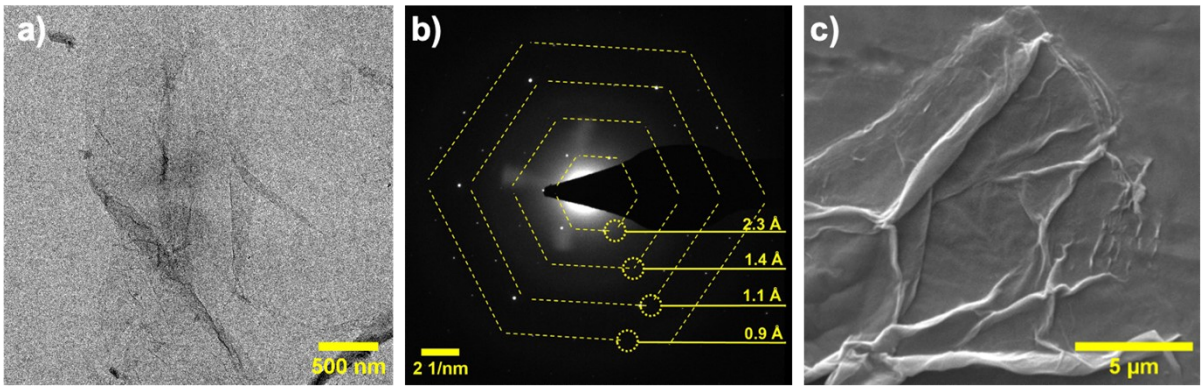

Figure S1: a) HR-TEM micrograph, b) SAED pattern and c) SEM micrograph (magnification: 8000x; scale bar: 5 µm) of GO

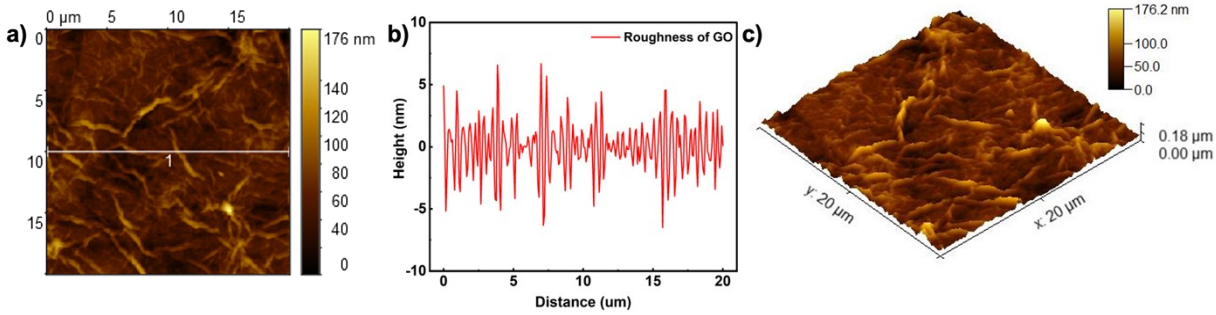

Figure S2: a) AFM image, b) roughness profile and c) three-dimensional topography by tapping mode (dimension 20 x 20 µm) of GO nanosheet

Table S1: Parameters related to roughness profile of GO nanosheet derived by AFM

Parameters	Values
Roughness average (R_a)	1.57 nm
Root mean square roughness (R_q)	2.11 nm
Maximum height of the roughness (R_t)	13.18 nm
Maximum roughness valley depth (R_v)	6.50 nm
Maximum roughness peak height (R_p)	6.68 nm
Average maximum height of the roughness (R_{tm})	10.86 nm
Average maximum roughness valley depth (R_{vm})	5.41 nm
Average maximum roughness peak height (R_{pm})	5.18 nm

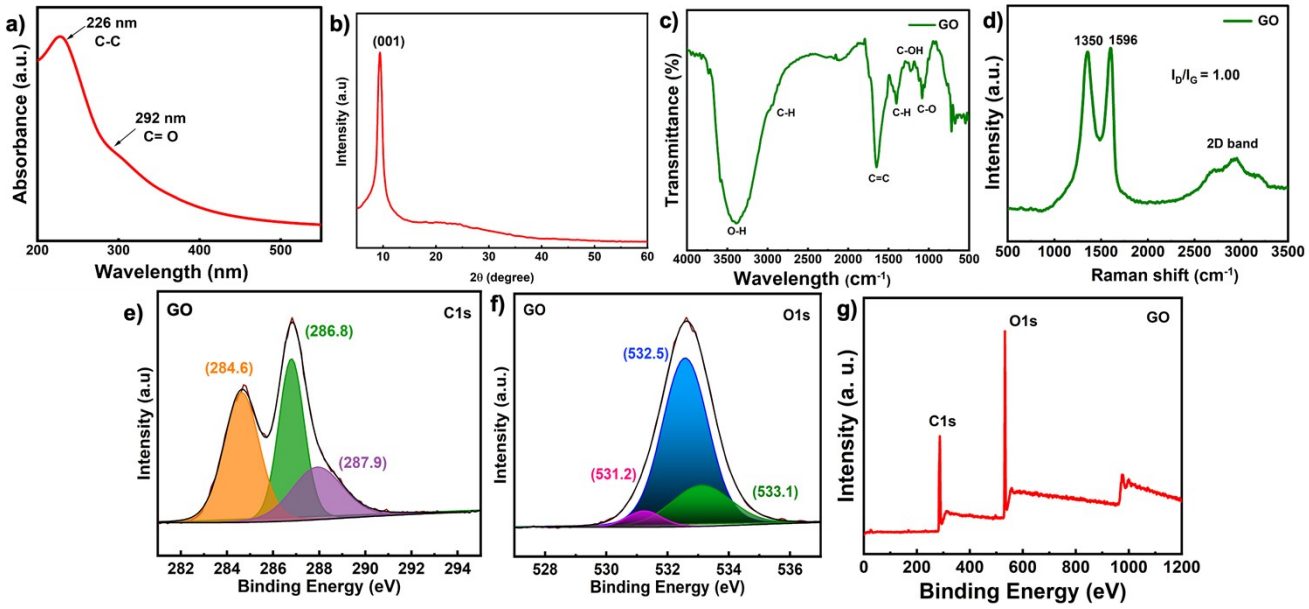

Figure S3: a) UV-visible, b) XRD, c) FTIR, d) Raman, e) deconvoluted spectra of C1s, f) deconvoluted spectra of O1s and g) wide spectra of XPS of GO

2. Effect of different concentration of GO nanosheets on NAG1 cells

1% bacterial suspension of NAG1 cells was added to 30 mL of media containing GO (20 to 150 µg/mL) for 12 h at 37 ^{o}C under shaking condition (120 rpm). 1 mL samples were withdrawn at every 3 hourly basis and analyzed for growth at 600 nm. Media with bacterial cells without addition of GO was maintained as positive control. Experiment was conducted in triplicates with error bars represented as standard deviation. The results are represented as mean of triplicate experimental values and treated groups showed statistically significant difference from the control group (NAG1 cells) by Student's t-test.

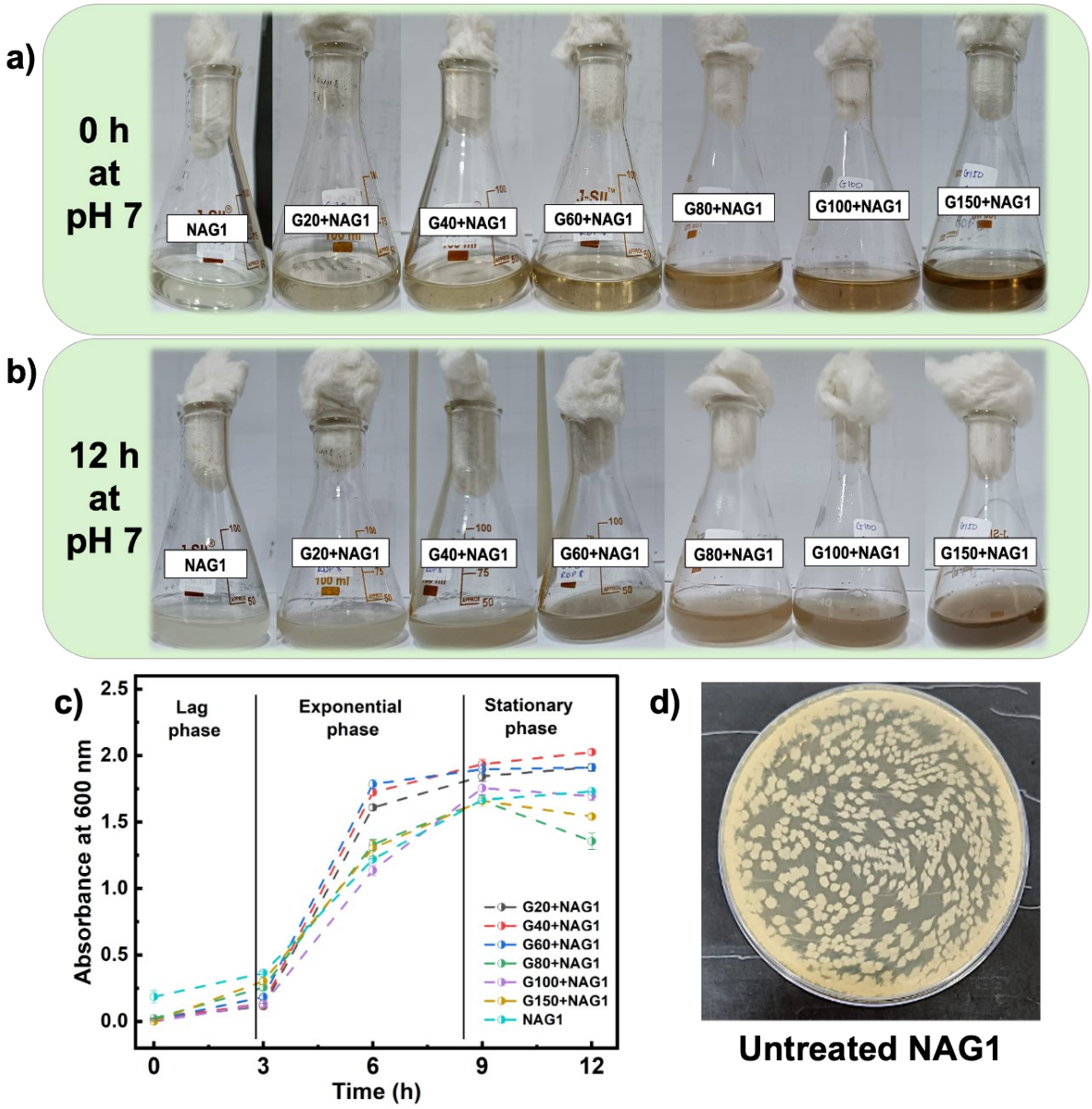

Figure S4: Images of flasks depicting NAG1 cells inoculated with G20 to G150 at a) 0 h and b) 12 h incubation, c) growth curve of NAG1 cells incubated with G20 to G150 at time interval 3, 6, 9 and 12 h and, d) mage of nutrient agar plate showing isolated colonies of marine bacteria NAG1 (untreated)

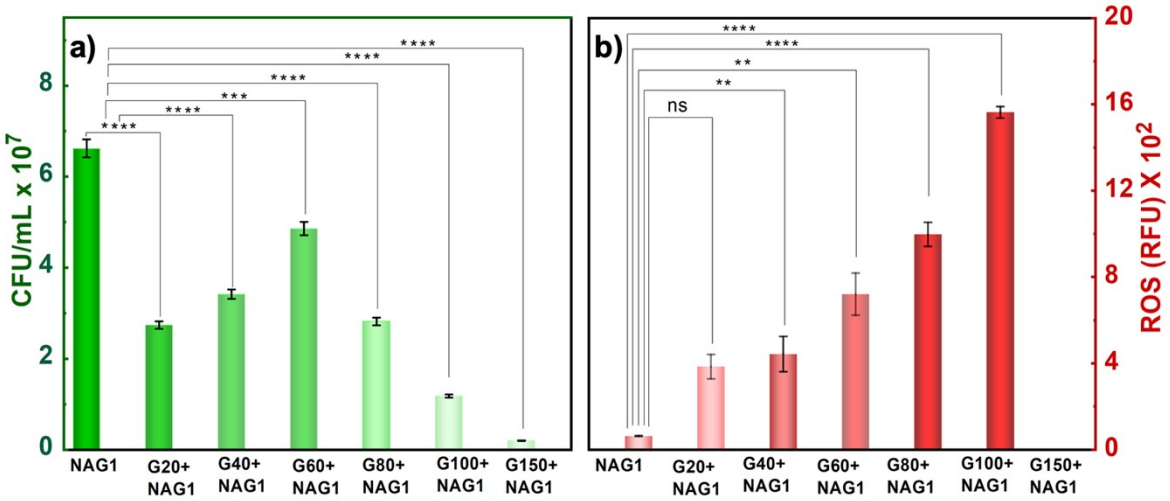

Figure S5: a) Bar graph showing cell viability expressed as colony forming unit per mL (CFU/mL) and b) ROS generation using DCFH-DA assay expressed as relative fluorescence unit with one-way ANOVA (ns: non-significant, *: p<0.05, **: p<0.01, ***: p<0.001 and ****: p<0.0001)

Table S2: Optimization of GO-NAG1 on 40,50 and 60 µgmL^{-1} at pH 4,7,11,14 with two-way ANOVA (ns: non-significant, *: p<0.05, **: p<0.01, ***: p<0.001 and ****: p<0.0001)

	Summary	P Value
pH4		
G40 vs. G50	ns	0.9971
G40 vs. G60	ns	0.9885
G50 vs. G60	ns	0.9971
pH7		
G40 vs. G50	****	<0.0001
G40 vs. G60	ns	0.7948
G50 vs. G60	****	<0.0001
pH9		
G40 vs. G50	****	<0.0001
G40 vs. G60	****	<0.0001
G50 vs. G60	****	<0.0001
pH11		
G40 vs. G50	****	<0.0001

G40 vs. G60	****	<0.0001
G50 vs. G60	****	<0.0001
pH14		
G40 vs. G50	****	<0.0001
G40 vs. G60	****	<0.0001
G50 vs. G60	ns	0.8695
G40		
pH4 vs. pH7	****	<0.0001
pH4 vs. pH9	****	<0.0001
pH4 vs. pH11	ns	0.1057
pH4 vs. pH14	ns	0.8769
pH7 vs. pH9	ns	0.1357
pH7 vs. pH11	****	<0.0001
pH7 vs. pH14	****	<0.0001
pH9 vs. pH11	****	<0.0001
pH9 vs. pH14	****	<0.0001
pH11 vs. pH14	ns	0.4435
G50		
pH4 vs. pH7	****	<0.0001
pH4 vs. pH9	****	<0.0001
pH4 vs. pH11	****	<0.0001
pH4 vs. pH14	****	<0.0001
pH7 vs. pH9	****	<0.0001
pH7 vs. pH11	****	<0.0001
pH7 vs. pH14	****	<0.0001
pH9 vs. pH11	****	<0.0001
pH9 vs. pH14	****	<0.0001
pH11 vs. pH14	****	<0.0001
G60		
pH4 vs. pH7	****	<0.0001
pH4 vs. pH9	****	<0.0001
pH4 vs. pH11	****	<0.0001
pH4 vs. pH14	****	<0.0001
pH7 vs. pH9	****	<0.0001
pH7 vs. pH11	****	<0.0001
pH7 vs. pH14	****	<0.0001
pH9 vs. pH11	****	<0.0001
pH9 vs. pH14	****	<0.0001

pH11 vs. pH14	***	0.0003

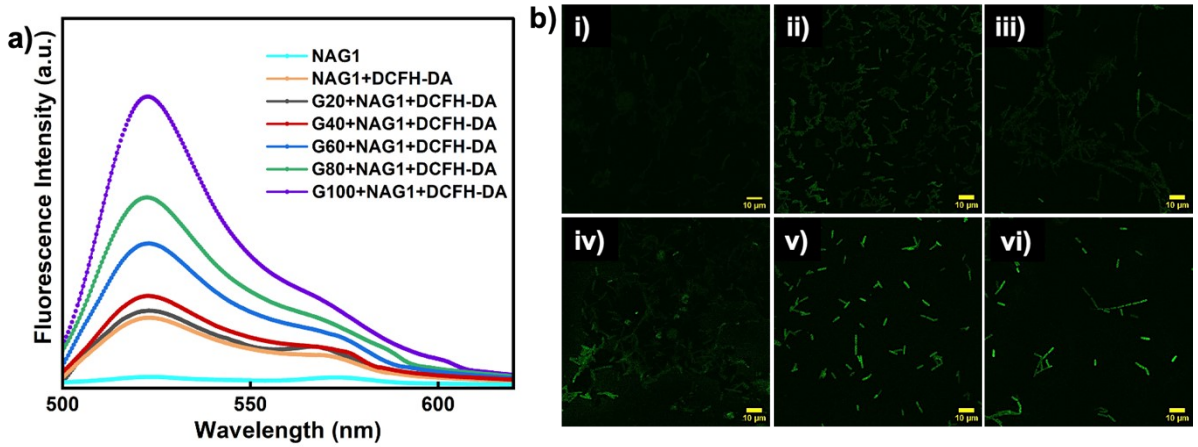

Figure S6: a) Fluorescence spectra of DCFH-DA at 522 nm of NAG1 after GO exposure, b) LSM images of NAG1 treated with varied GO concentrations after DCFH-DA assay: i) NAG1 (untreated), ii) G20+NAG1, iii) G40+NAG1, iv) G60+NAG1, v) G80+NAG1 and vi) G100+NAG1 (scale bar: 10 µm)

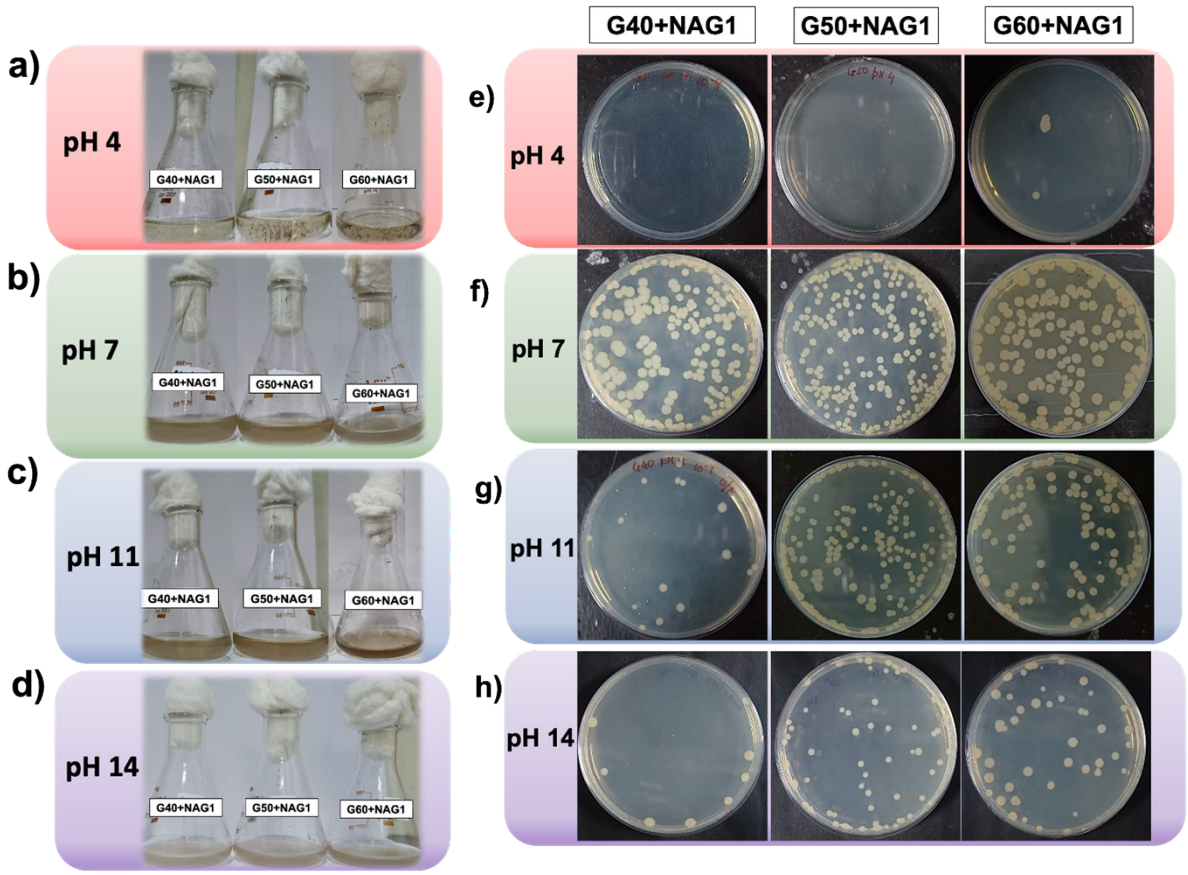

Figure S7: Images of flasks containing NAG1 incubated in G40, G50 and G60 at a) pH 4, b) pH 7, c) pH 11 and d) pH 14. Images of nutrient agar plated with serially diluted 50 µL sample of e) pH 4, f) pH 7, g) pH 11 and h) pH 14 incubated for 24 h

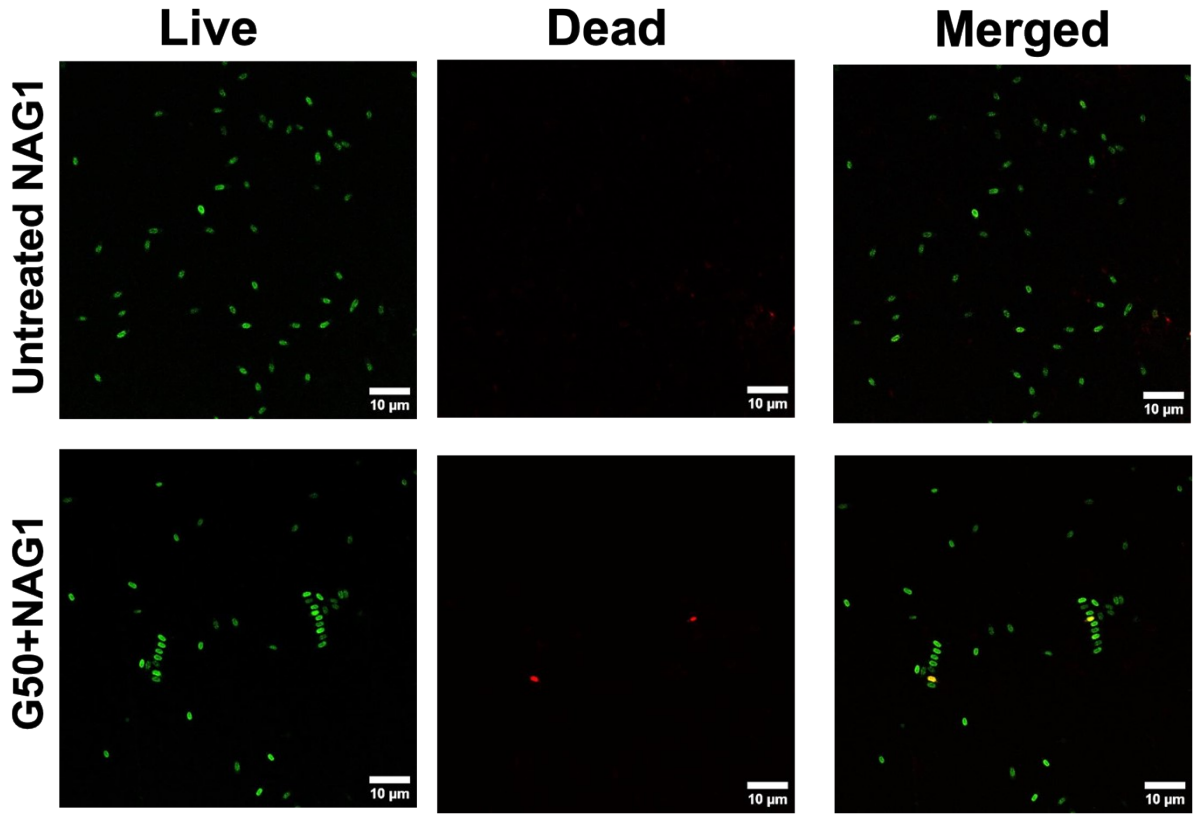

Figure S8: LSM images of live and dead assay of untreated NAG1 (control) and G50+NAG1 (scale bar: 10 µm)

Preparation of ESEM for bacterial sample

1 mL of each sample was centrifuged at 8000 rpm for 5 minutes to pellet down the bacteria. The pellet was washed thrice with sterile distilled water at 5000 rpm for 5 minutes. The bacterial cells were fixed using 4% paraformaldehyde and incubated for 30 minutes at 4 ˚C. After incubation, cells were serially dehydrated using 30, 50, 70, 90 and 100% ethanol.

3. Topographical changes by AFM analysis

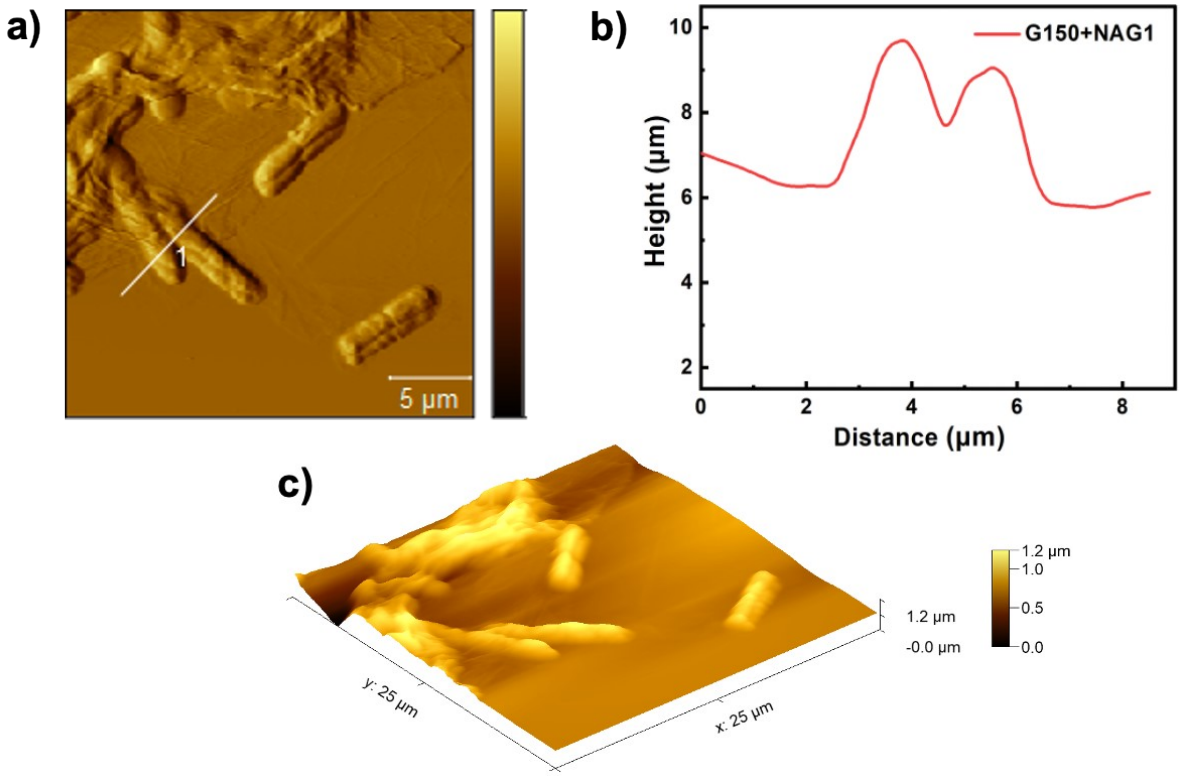

Figure S9: a) AFM image of G150+NAG1, b) roughness profile of G150+NAG1 and c) three-dimensional topography of G150+NAG1 (dimension 20 x 20 µm)

Table S3: Parameters related to roughness profile of untreated NAG1 and GO-incubated cells derived by AFM

Parameters	NAG1	G50+NAG1	G150+NAG1
Roughness average (R_a)	4.6 nm	4.3 nm	1.9 nm
Root mean square roughness (R_q)	6.7 nm	5.6 nm	2.6 nm
Maximum height of the roughness (R_t)	47.8 nm	35.0 nm	17.2 nm
Maximum roughness valley depth (R_v)	26.9 nm	19.5 nm	6.8 nm
Maximum roughness peak height (R_p)	20.9 nm	12.4 nm	10.4 nm
Average maximum height of the roughness (R_{tm})	23.8 nm	23.8 nm	9.8 nm

Average maximum roughness valley depth (R_{vm})	12.3 nm	13.3 nm	4.3 nm
Average maximum roughness peak height (R_{pm})	11.0 nm	10.5 nm	5.4 nm

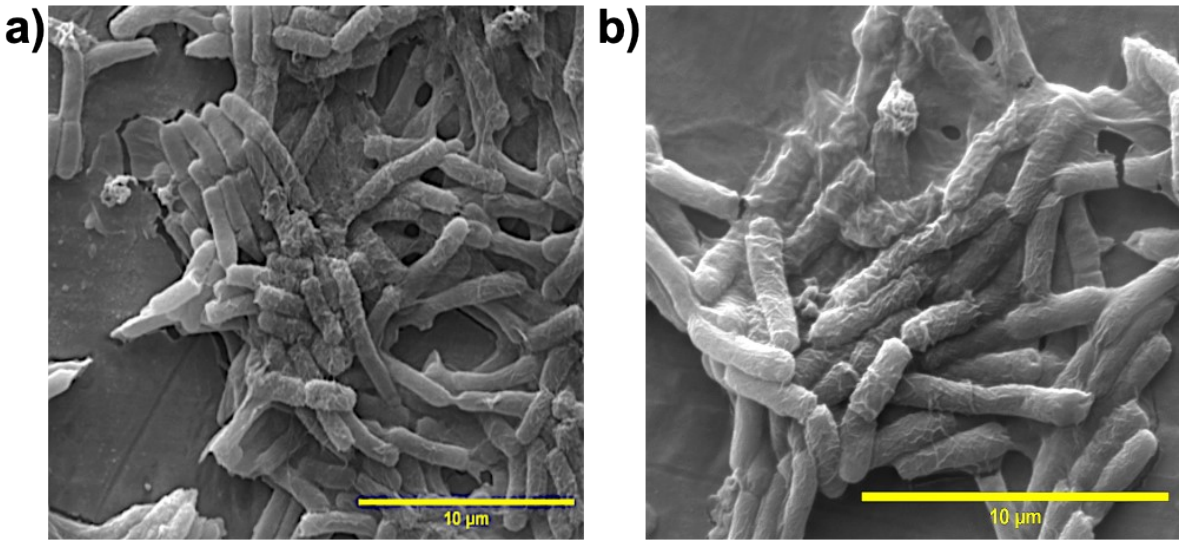

Figure S10: ESEM micrographs of a) G100+NAG1 (magnification: 7000x) and b) G150+NAG1 (magnification: 7000x) after 12 h incubation (scale bar: 10 µm)

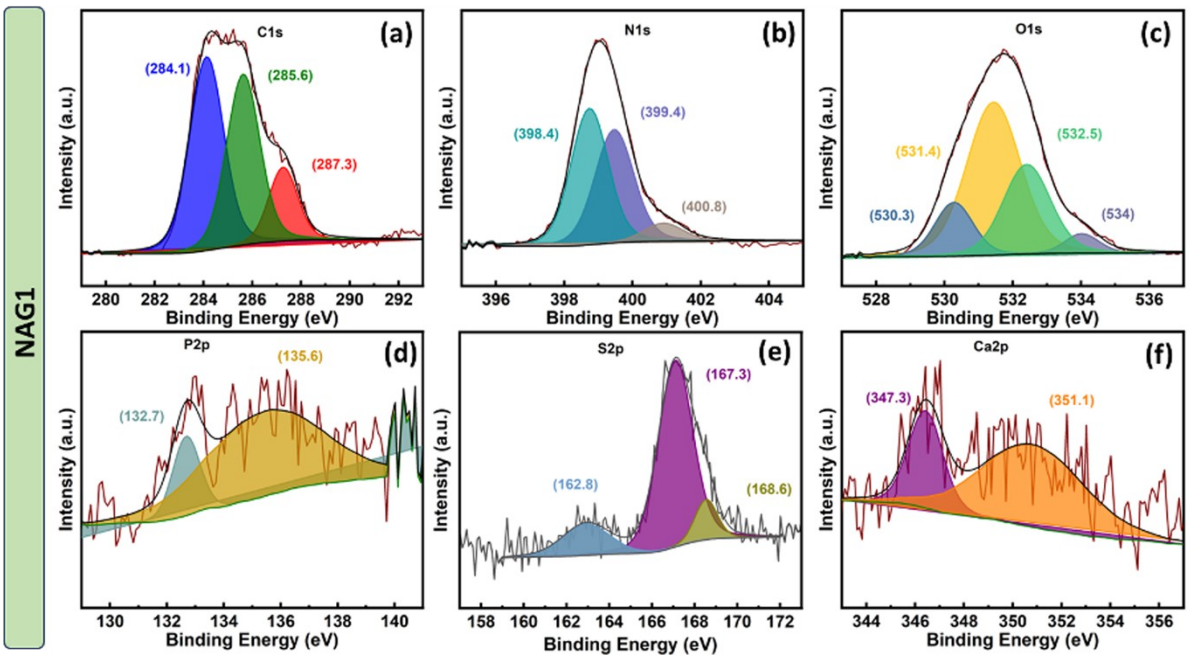

Figure S11: Deconvoluted XPS spectra of NAG1: a) C1s, b) N1s, c) O1s, d) P2p, e) S2p, and f) Ca2p

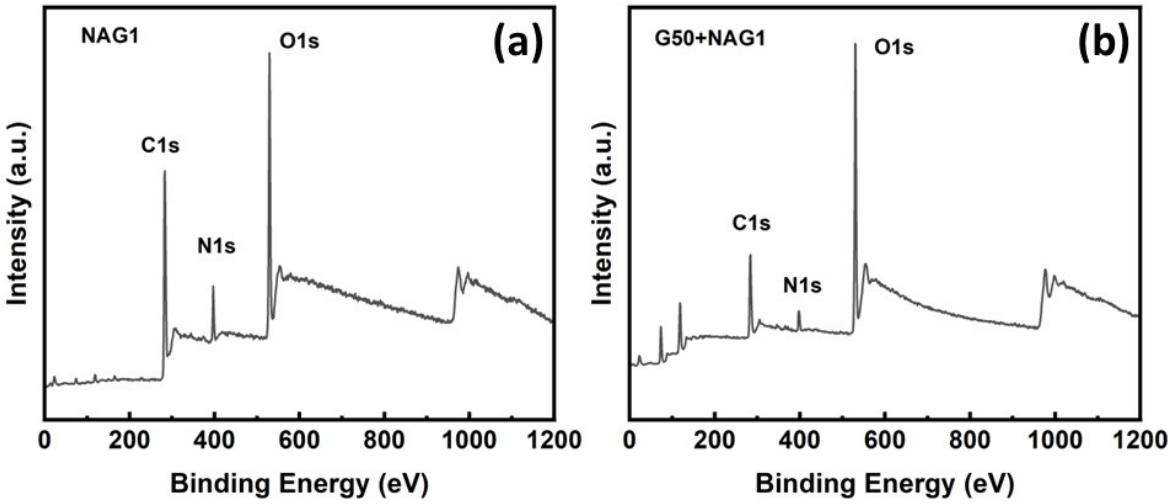

Figure S12: Wide spectra of (a) NAG1 and (b) G50 + NAG1

4. Dye removal assay

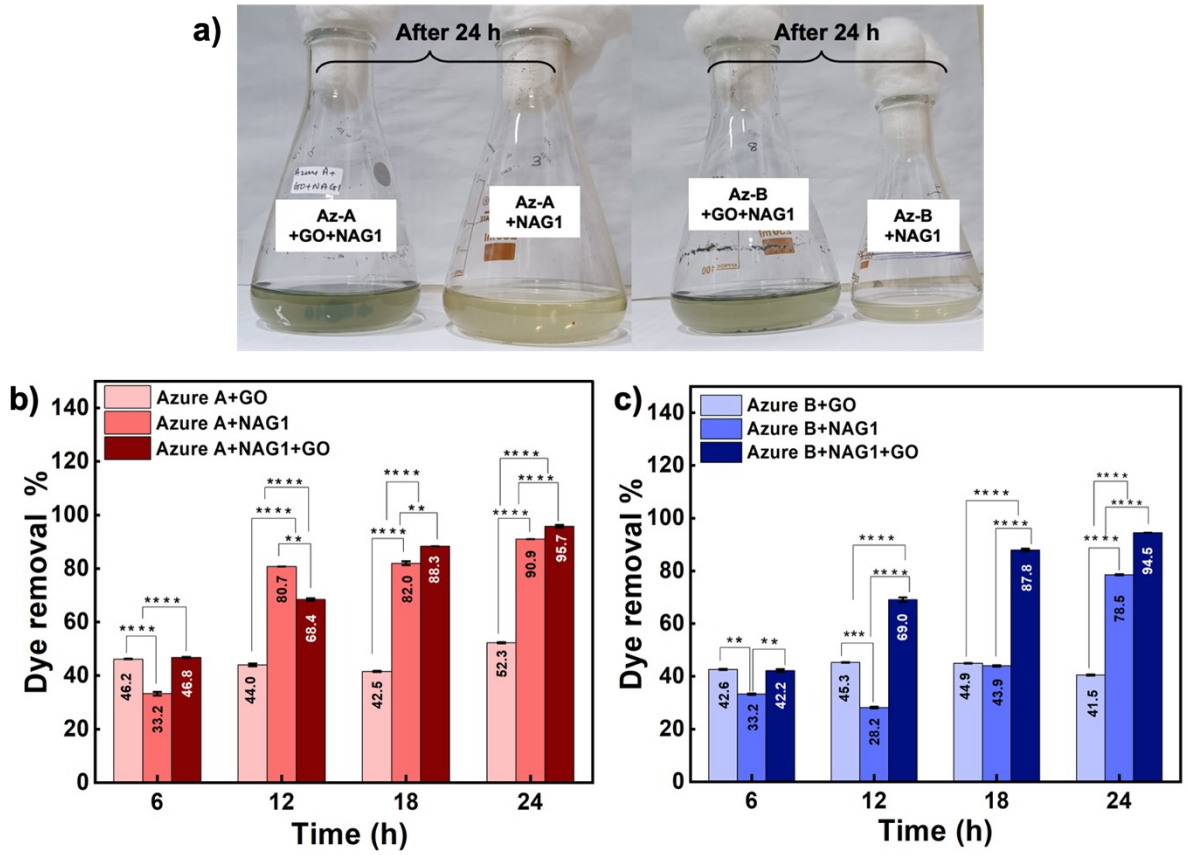

Figure S13: a) Image of Az-A and Az-B dye removal by broth assay after 24 h incubation, Dye removal using GO, NAG1 and GO-NAG1 of b) Az-A and c) Az-B with statistical analysis

using two-way ANOVA (ns: non-significant, *: p<0.05, **: p<0.01, ***: p<0.001 and ****: p<0.0001)

Table ST4: Abundance of degraded intermediates detected in LC-MS

Time (Minutes)	Peaks (m/z ratio)	Samples		
		Untreated Az-A	Az-A+NAG1	Az-A+NAG1+GO
21.9	301.1	--	380349.9	381556.3
	149.0	--	422388.9	185588.3
19.3	200.1	150736.8	--	--
	124.0	58966.8	--	--
18.4	293.0	--	137882.3	--
	124.08	--	45841.1	--
17.5	415.2	--	90785.02	--
	124.0	--	42143.97	--
14.6	270.2	10242122	--	1432872.2
9.5	270.1	--	--	128159.23
	218.2	--	--	76060.6
7.8	439.2	386439.31	--	--
5.5	197.1	--	448539.7	240925.3
4.7	229.1	100819.7	--	--
	189.0	55690.1	--	--
3.2	166.0	1259217.5	--	--
	120.07	5866359	--	--
	100.0	--	4037859.7	3509773.5
2.4	254.1	483093.7	424998.8	434960.7
	155.0	707656.44	193202.7	167166.3

1.8	235.1	--	436403.0	193155.1
	187.1	--	457944	--
	137.0	--	340582.2	433735.1
Time (Minutes)	**Peaks (m/z ratio)**	**Samples**		
		Untreated Az-B	**Az-B+NAG1**	**Az-B+NAG1+GO**
14.5	270.2	10104213	5818454.5	5993489.5
7.8	439.2	311134.72	--	--
	245.1	74665.98	--	--
5.6	197.2	--	264715.41	283158.7
4.7	448.1	--	225333.7	127185.3
	284.1	--	--	14935.1
3.2	166.0	661175.1	--	--
	120.0	4163984.5	--	--
	100.0	1803182.6	3248570.25	3238806.2
2.4	254.1	453008.31	3248570.25	3238806.25
	155.0	159340.0	383795.4	349822.97
1.9	268.1	213323.0	--	--
	235.1	---	398649.2	354960.8
	226.1	136332.0	--	--
	137.0	--	344690.63	689935.94
	121.0	185408.3	--	--
1.7	187.1	--	210945.3	61537.1

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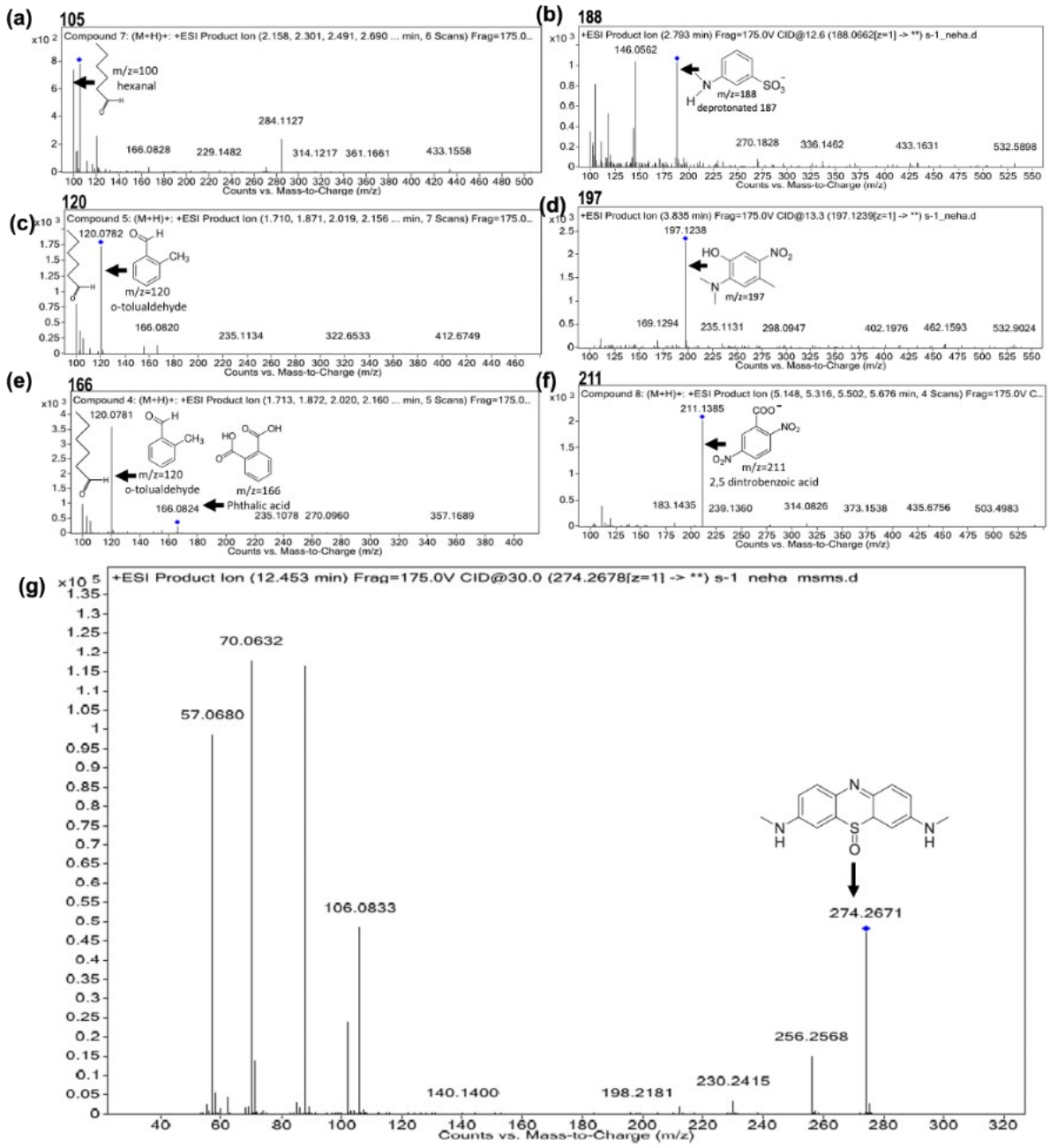
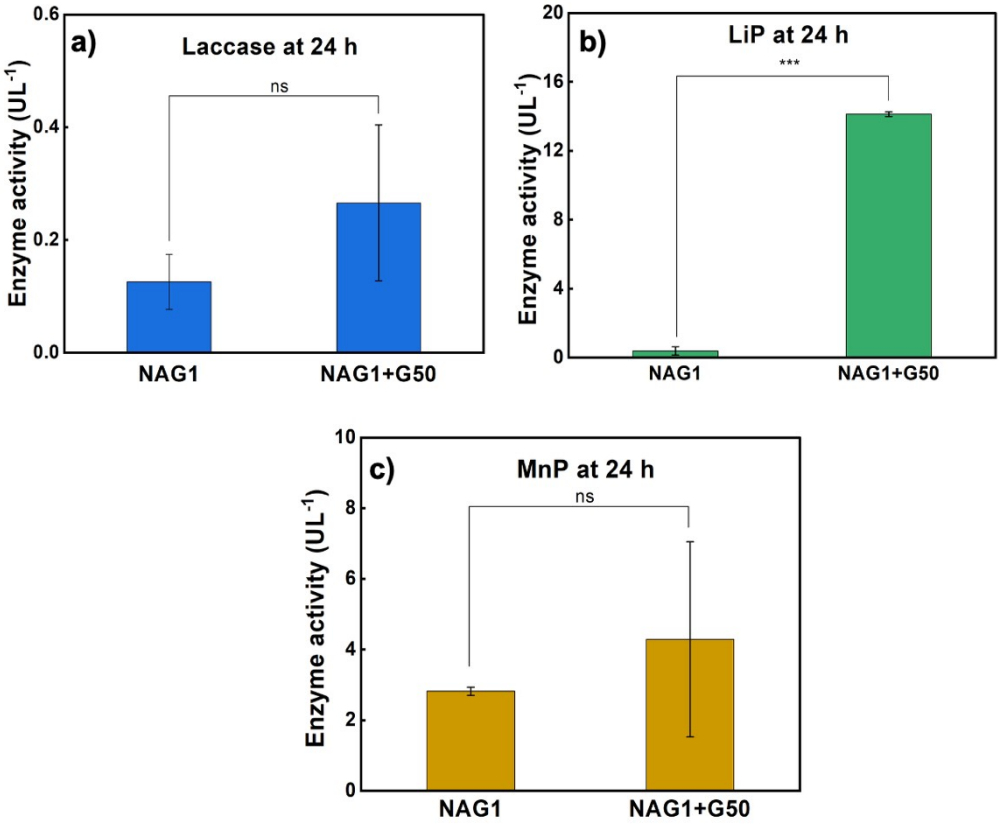


Figure S14: Tandem mass spectrometry scans for (a) m/z = 105, (b) m/z = 188, (c) m/z = 120, (d) m/z = 197, (e) m/z =166, (f) m/z = 211 and (g) tandem MS scan of various species



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138 **Figure S15:** Screening of ligninolytic enzymes present in NAG1 and NAG1+G50

139 a) Laccase (Lac), b) Lignin Peroxidase (LiP) and c) Manganese Peroxidase (MnP)

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