

Electronic Supplementary Information

A chemo-microbial hybrid process for the production of 2-pyrone-4,6-dicarboxylic acid as a promising bioplastic monomer from PET waste

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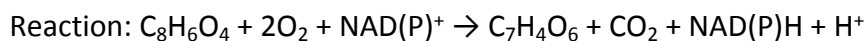
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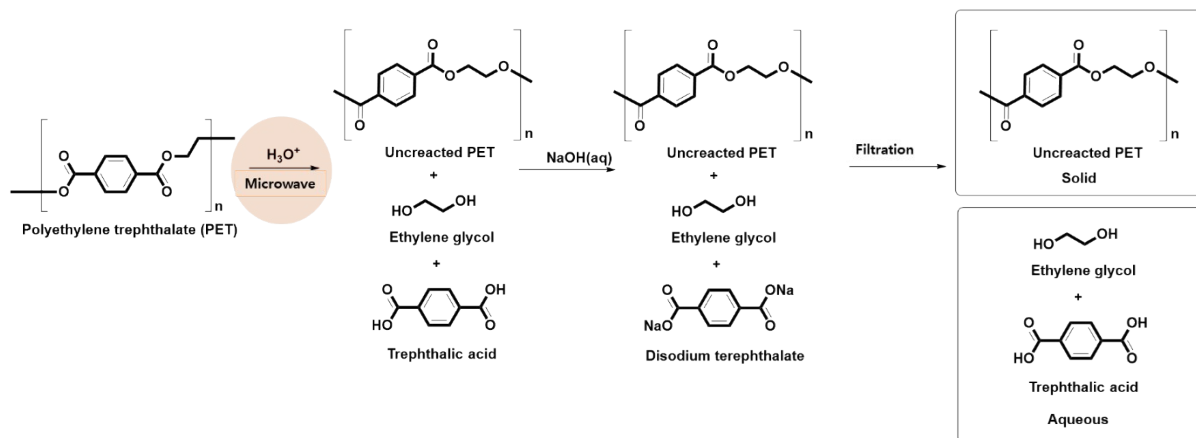
Calculation of PDC conversion yield

$$PDC \text{ Conversion yield (\%)} = \frac{\text{Produced PDC } \left(\frac{g}{L}\right)}{\text{Initial TPA } \left(\frac{g}{L}\right)} \times \text{Conversion factor } (C_f) \times 100$$



$$\text{Conversion factor } (C_f) = \frac{1 \text{ mol of PDC}}{1 \text{ mol of TPA}} = \frac{184.1}{166.13} = 1.11$$

Where, conversion factor (C_f) is theoretical maximal value for the conversion of TPA to PDC considering molecular weight change by enzymatic reactions (LigABC). Because TPA can be converted to PDC without by-pathway, conversion factor is obtained based on molecular weight of TPA and PDC.



Scheme S1. Overall reaction scheme of PET hydrolysis.

Table S1. Plasmids and bacterial strains used in this study.

Strain and plasmid	Relevant characteristic	Reference or source
Strain		
<i>E. coli</i> XL1-Blue	<i>recA1 endA1 gyrA96 thi-1 hsdR17 supE44 relA1 lac</i> [F' <i>proAB lacI^qΔM15 Tn10</i> (Tet ^R)]	Stratagene
Strains		
<i>E. coli</i> CHMS	<i>E. coli</i> XL1-Blue with pKE112 ligAB	this study
<i>E. coli</i> PDC _{PCA}	<i>E. coli</i> XL1-Blue with pKE112 ligABC	this study
<i>E. coli</i> PDC _{TPA}	<i>E. coli</i> XL1-Blue with pKE112tphB ligABC and pKM212 tphAabc	this study
<i>E. coli</i> PCA	<i>E. coli</i> XL1-Blue with pKE112 tphB and pKM212 tphAabc	this study
Plasmid		
pKE112 ligAB	pKE112; P _{tac} promoter, <i>Sphingomonas paucimobilis</i> strain <i>ligAB</i> gene, Amp ^R	this study
pKE112 ligABC	pKE112; P _{tac} promoter, <i>Sphingomonas paucimobilis</i> strain <i>ligABC</i> gene, Amp ^R	this study
pKE112 tphB	pKE112; P _{tac} promoter, <i>Comamonas</i> sp. strain E6 <i>tphB</i> genes, Amp ^R	this study
pKM212 tphAabc	pKM212; P _{tac} promoter, <i>Comamonas</i> sp. strain E6 <i>tphAabc</i> genes, Km ^R	this study
pKE112 tphBligABC	pKE112; P _{tac} promoter, <i>Comamonas</i> sp. strain E6 <i>tphB</i> gene, <i>Sphingomonas paucimobilis</i> strain <i>ligABC</i> gene, Amp ^R	this study

Table S2. Gene information used in this study.

Gene	UniProtKB Accession number	Sequence
<i>tphAa</i>	Q3C1D2	<p>ATGAACCACCAGATCCATATCCACGACTCCGATATCGCGTTCCTGCGCGCCCGGGCA ATCCGTACTGGATGCAGCTCTGCAGGCCGGCATCGAGCTGCCCTATTCCTGCCGCAAAG GTAGCTGTGGCAACTGTGCGAGTACGCTGCTCGACGAAAATTGCCTCCTCAATGGC ATGGCCGTGCGAAACGAACTCTGCGCCTCGGAACAAGTGCTGCTGTGCGGCTGCACTG CAGCCAGCGATATACGTATCCACCCGAGCTCCTTTCGCCGTCTCGACCCGGAAGCCCGA AAACGTTTTACGGCCAAGGTGTACAGCAATACACTGGCGGCACCCGATGTCTCGCTGCT GCGCCTGCGCCTGCCTGTGGGCAAGCGCGCAAATTTGAAGCCGGCCAATACCTGCTG ATTCACCTCGACGACGGGGAAAGCCGCAGCTACTCTATGGCCAATCCACCCCATGAGA GCGATGGCATCACATTGCATGTCAGGCATGTACCTGGTGGTTCGCTTCAGCACTATCGTT CAGCAGTTGAAGTCTGGTGACACATTGGATATCGAACTGCCATTCGGCAGCATCGCACT GAAGCCTGATGACGCAAGGCCCTGATTTGCGTTGCGGGTGGCACGGGATTTGCGCCC ATTAATCCGTTCTTGATGACTTAGCCAAACGCAAGGTTAGCGCGACATCACGCTGAT CTGGGGGGCTCGCAACCCCTCGGCCTGTATCTTCTAGCGCCATCGACAAGTGGCGC AAAGTCTGGCCACAGTTTCGCTACATTGCAGCCATCACCGACCTAGGCGATATGCCTGC GGATGCTCACGCAGGTGCGGTGGATGACGCGCTACGCACTACTTTGGCAACCTGCAC GATCATGTGGTGCCTGCTGTGGCTCACCAGCTCTGGTTCAATCAGTGCGCACAGCCGC TTCCGATATGGGCCTGCTTGACAGGACTTCCACGCGGATGTTTTTTCGACAGGCCCGA CTGGTCACCACTAG</p>
<i>tphAb</i>	Q3C1D5	<p>ATGCAAGAATCCATCATCCAGTGGCATGGGGCCACTAATACGCGCGTGCCTTTTGGTAT CTATACCGACACAGCCAATGCTGATCAGGAACAGCAGCGCATCTATCGCGGCGAGGTC TGGAATACTTGTGCCTGGAATCTGAAATTCCTGGGGCCGGTGATTTCCGCACTACCTT TGCCGGTGAAACACCGATAGTTGTCGTACGGGATGCCGACCAGGAAATCTACGCCTTC GAGAACCCTGCGCGCATCGCGGCTCTCATCGCTCTGGAGAAATCGGGCCGTACGG ATAGTTTCCAGTGCCTATCACGCCTGGAGCTACAACCGACAGGGAGATCTGACCGG CGTTGCCTTCGAGAAAGGTGTCAAGGGCCAGGGTGGCATGCCGGCCTCATTCTGCAAA GAAGAGCATGGCCCGCGCAAGCTCCGCGTGGCTGTCTTTTTCGGTTTGGTCTTTGGCA GTTTTTCCGAGGACGTGCCAGCATTGAGGATTACCTTGGCCCTGAGATTTGCGAGCGC ATAGAGCGCGTGTGCACAAGCCGTAGAAGTCATCGGTCGCTTTCACGAAAAGCTGC CTAACAAGTGAAGCTCTACTTCGAGAACGTGAAGGACAGCTATCACGCCAGCCTCTG CATATGTTCTTACCACCTTCGAGCTGAATCGCCTCTCACAAAAGGCGGTGCATCGTC</p>

		GACGAGTCGGGTGGCCACCATGTGAGCTATTCCATGATCGATCGCGGCGCCAAAGACG ACTCGTACAAGGACCAGGCCATCCGCTCCGACAACGAGCGTTACCGGCTCAAAGATCC TAGCCTTCTAGAGGGCTTTGAGGAGTTCGAGGACGGCGTGACCTGCAGATCCTTTCT GTGTTCCCTGGCTTTGTGCTGCAGCAGATTGAGAACAGCATCGCCGTGCGTCAGTTGCT GCCCCAAGAGCATCTCCAGCTCGGAACTCAACTGGACCTATCTTGGCTATGCAGATGACA GTGCCGAGCAACGCAAGGTCAGACTCAAACAGGCCAACCTTATCGGCCCGGCCGGATT CATTTCCATGGAAGACGGAGCTGTGCGTGGATTCTGTCAGCGTGGCATCGCAGGCGCT GCCAACCTTGATGCAGTCATCGAGATGGGCGGAGACCACGAAGGCTCTAGCGAGGGC CGCGCCACGGAAACCTCGGTACGCGGCTTTTGAAGGCCTACCGCAAGCATATGGGAC AGGAGATGCAAGCATGA
<i>tphAc</i>	Q3C1D4	ATGATCAATGAAATTCAAATCGCGGCCTTCAATGCCGCCTACGCGAAGACCATAGACA GTGATGCAATGGAGCAATGGCCAACCTTTTTACCAAGGATTGCCACTATTGCGTCACC AATGTCGACAACCATGATGAGGGACTTGTGCCGGCATTGTCTGGGCGGATTGCGCAGG ACATGCTCACCAGCGAATTTCTGCGCTGCGGAAGCCAATATCTACGAGCGCCACCGC TATCGCCATATCCTGGGTCTGCCTTCGATCCAGTCAGGCGATGCAACACAGGCCAGCGC TTCCACTCCGTTTCATGGTGCTGCGCATCATGCATACAGGGGAAACAGAGGTCTTTGCCA GCGGTGAGTACCTCGACAAATTCACCACGATCGATGGCAAGTTACGTCTGCAAGAACG CATCGCGGTTTTCGACAGCAGGTGACGGACACGCTGATGGCATTGCCGCTATGA
<i>tphB</i>	Q3C1D3	ATGACAATAGTGCACCGTAGATTGGCTTTGGCCATCGGCGATCCCCACGGTATTGGCCC AGAAATCGCACTGAAAGCTCTCCAGCAGCTGTCTGTCACCGAAAGGTCTCTTATCAAGG TCTATGGACCTTGAGCGCTCTCGAGCAAGCCGCACGGGTTTTCGAAATGGAGCCGCT TCTTCAAGACATCGTTCACGAGGAAGCCGGCACACTTACACAACCAAGTTCAATGGGGA GAAATCACCCCGCAGGCTGGTCTATCTACGGTGCAATCCGCAACAGCGGCTATCCGAG CGTGCGAAAACGGCGAAGTCGATGCCGTCATTGCCTGCCCTACCATGAAACGGCCAT TCACCGCGCAGGCATAGCGTTCAGCGGCTACCCATCTTTGCTCGCCAATGTTCTTGGA TGAACGAAGACCAGGTATTCTGATGCTGGTGGGGGCTGGCCTGCGCATAGTGCATGT CACTTTGCATGAAAGCGTGCGCAGCGCATTGGAGCGGCTCTCTCCTCAGTTGGTGGTCA ACGCGGCGCAGGCTGCCGTGCGACATGCACCTTACTCGAGTGCCTAAACCAAAAAGT CGCTGTATTCGGGATCAACCCTCATGCATCTGAAGGACAGTTGTTCCGGCCTGGAGGACT CCCAGATCACCGTTCGCTGTGCGAGACTGCGCAAGCGCGGCTAGCAGTAGACGG CCCCATGGGAGCTGACATGGTTCTGGCACAGCGCAAGCACGACCTGTATGTAGCCATG CTGCACGATCAGGGCCATATCCCCATCAAGCTGCTGGCACCTAACGGAGCCAGCGCAC TATCTATCGGTGGCAGGGTGGTGCTTTCCAGCGTGGGCCATGGCAGCGCCATGGACAT TGCCGGCCGTGGCGTGGCTGACGCCACGGCCCTCTACGCACAATAGCCCTACTCGGA GCCAACCGGTCTGA
<i>ligA</i>	P22635	ATGACCGAGAAGAAAGAGAGAATCGACGTTACGCCTATCTCGCCGAGTTTGACGACA TTCCCGCACCCCGTGTTCACCGCCAGCGCGCGCAAGGGCTATAATCTCAACCA GTTCCGCGATGAGCCTGATGAAGGCCGAGAACCGCGAGCGGTTCAAGGCCGACGAGAG

		CGCCTATCTGGACGAGTGGAACTCACGCCCGCCGCAAGGCCGCGTGTGGCCCCG GACTACAATGCGATGATCGACGAGGGCGGGAATGTCTATTTCTGTCCAAGCTGTTCTC GACCGACGGCAAGAGCTTCCAGTTCCGCCGCGGCTCGATGACCGGCATGACTCAGGAA GAATATGCACAGATGATGATCGATGGCGGCCGTTCCGCCGCGGGTGTGCGCTCGATCA AGGGAGGCTACTGA
<i>ligB</i>	P22636	ATGGCACGCGTTACCACGGGCATCACGTCCAGCCACATTCGCGCTCGGCGCGGCCA TCCAGACCGGCACCAGCGACAATGATTACTGGGGCCCGGTGTTCAAGGGCTACCAGCC GATCCGCGACTGGATCAAGCAGCCCGCAACATGCCGGACGTCGTGATCCTGGTCTAT AACGACCATGCCTCGGCCTTGCAGATGAACATCATCCCGACCTTCGCGATCGGCTGCGC GGAAACGTTCAAGCCC GCCGACGAGGGATGGGGCCCGCGCCCGGTGCCGACGTGAA GGGCCATCCGACCTTGCTGGCACATCGCCAGAGCTGATCCTCGACGAGTTGAC ATGACCATCATGAACCAGATGGACGTCGATCATGGCTGCACCGTGCCGCTCTCGATGAT CTTCGGCGAGCCCGAGGAATGGCCGTGCAAGGTATCCCTTCCCGGTCAATGTCGTC ACTTATCCGCCGCGTCCGGCAAGCGCTGCTTCGCGCTCGGTGACAGCATCCGCGCCG CGGTGAGAGCTTCCCGAAGACCTCAACGTCCATGTCTGGGGCACCGGCGGCATGAG CCACCAGCTTCAGGGCCCGCGCGCCGGCCTCATCAACAAGGAGTTCGACCTGAACTTCA TCGACAAGCTGATCAGCGACCCCGAGGAGCTGAGCAAGATGCCGCACATCCAGTATCT GCGCGAAAAGCGGATCGGAAGGCATCGAGCTGGTCATGTGGTTCATCATGCGCGGCGC GCTGCCGAGAAGGTGCGGGATCTTACACCTTCTATCACATCCCGCCTCCAACCCG CGCTCGGCGGATGATCCTGCAGCCGAGGAGACCGCAGGTACACCGCTCGAACC GC GCAAGGTGATGAGCGGACACAGCCTGGCCAGGCCTGA
<i>ligC</i>	Q9KWL3	ATGAGAATAGCTCTCGCAGGCGCCGGCGCATTGCGCGAAAAACATCTCGACGGTCTCA AGAATATCGACGGCGTCGAGATCGTCTCGATCATCAGCCGCAAGGCCGAGCAGGCCGC GGAAGTCGCCGCAAATATGGCGCAAGCACAGCGGCACCGATCTTCCGAAGCGCTT GCCCCTGACGATGTCGACGCGGTGATCCTCTGCACCCACCCAGATGCATGCCGAGC AGGCCATTGCCTGCATGAATGCCGGCAAGCATGTCCAGGTGGAAATCCCGCTGGCGGA CAGCTGGGCCGATGCCAAGCGGTGATGAAGAAGAGCCAGGAAACCGGCCTCGTCTG CATGGTTCGGCCACACCCCGCTTCAACCCGAGCCACAGTATATCCACAACAAGATCG TGCCCGGCGAGCTCGCCATCCAGCAGATGGACGTGCAGACCTATTTCTCCGCCGCAA GAACATGAACGCCAAGGGCGAGCCGCGGTCTTGACGGACCATCTGCTCTGGACCAT GCCGCGCACACGGTGGACCTGTTGCTTATCAGGCCGGCAAGATCGTGCAGGCCAATG CCGTGCAGGGGCCGATCCATCCCGAGCTCGGCATCGCCATGGACATGTCGATCCAGCT CAAGAGCGAGACGGGCGCGATCTGCACCCTGTCCCTCTCGTTCAACAATGACGGGCCG CTCGGCACCTTCTCCGCTACATCTGCGACAACGGCACCTGGATCGCGCGCTACGACGA TCTGGTGACCGGCAAGGAAGAACCCGTGGACGTGAGCAAGGTGACGTGTCGATGAA CGGGATCGAGCTGCAGGATCGGGAATTCATCGCGGCGATCCGCGAAGGTGCGGAGCC CAATTCGTGCGGTGGCGAGAGTGCTCGATTGCTATCGGGTGTGGGCGAACTCGAGGTC CAGCTCGAAAAGCAGGGCTGA

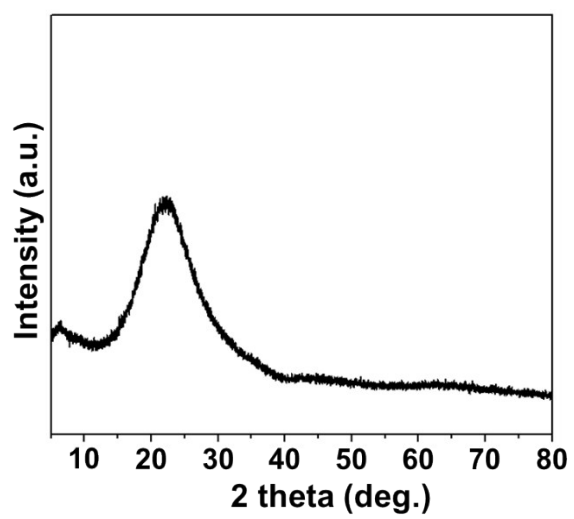


Fig. S1. XRD patterns of SiO₂-R catalyst.

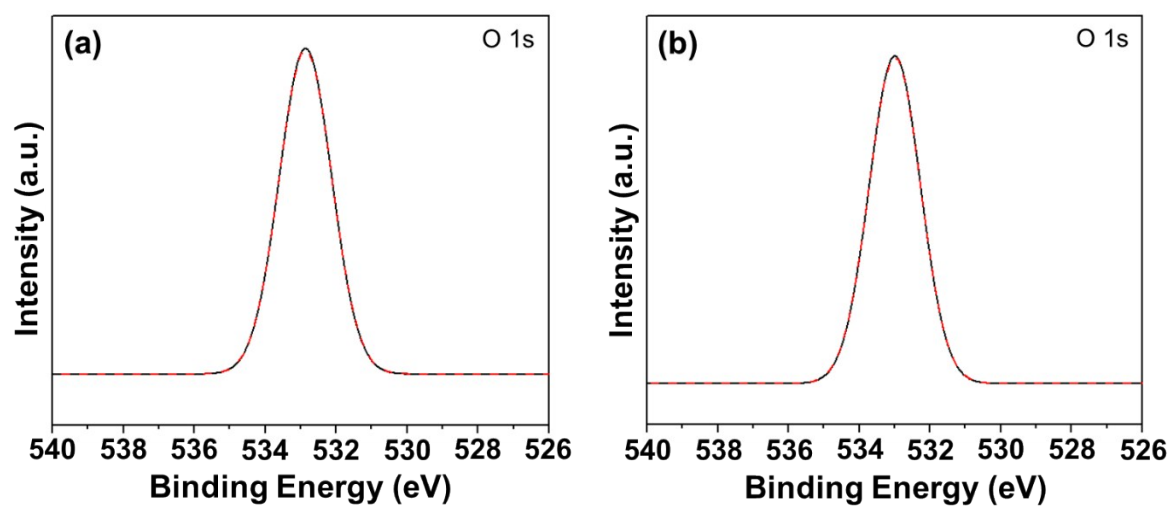


Fig. S2. O 1s XPS spectra of rice husk derived (a) SiO₂-R and (b) thiol-SiO₂.

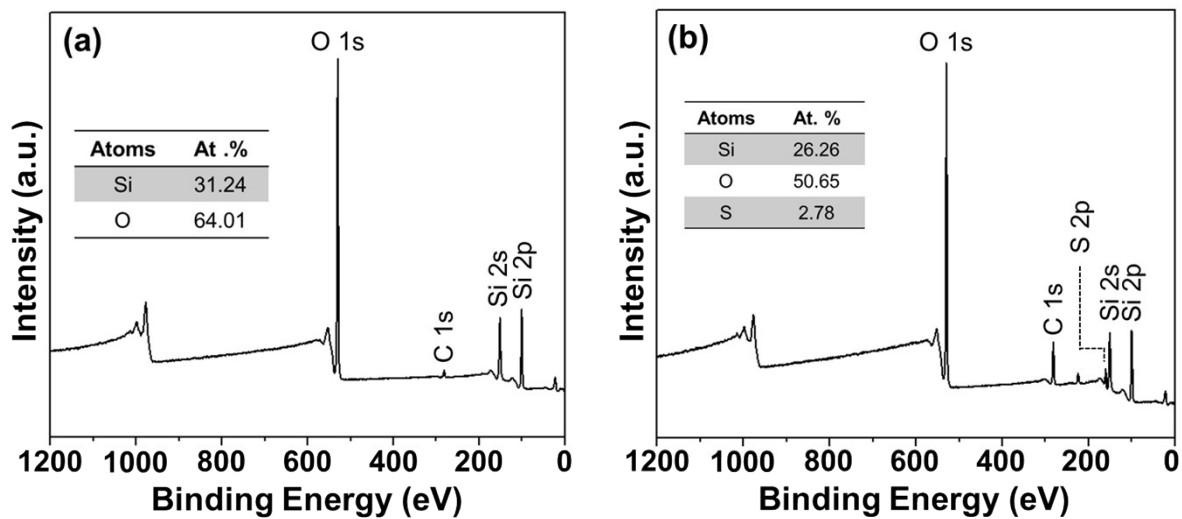


Fig. S3. XPS survey spectra of (a) rice husk derived $\text{SiO}_2\text{-R}$ and (b) thiol- SiO_2 (inset: atomic ratio of each atomic species).

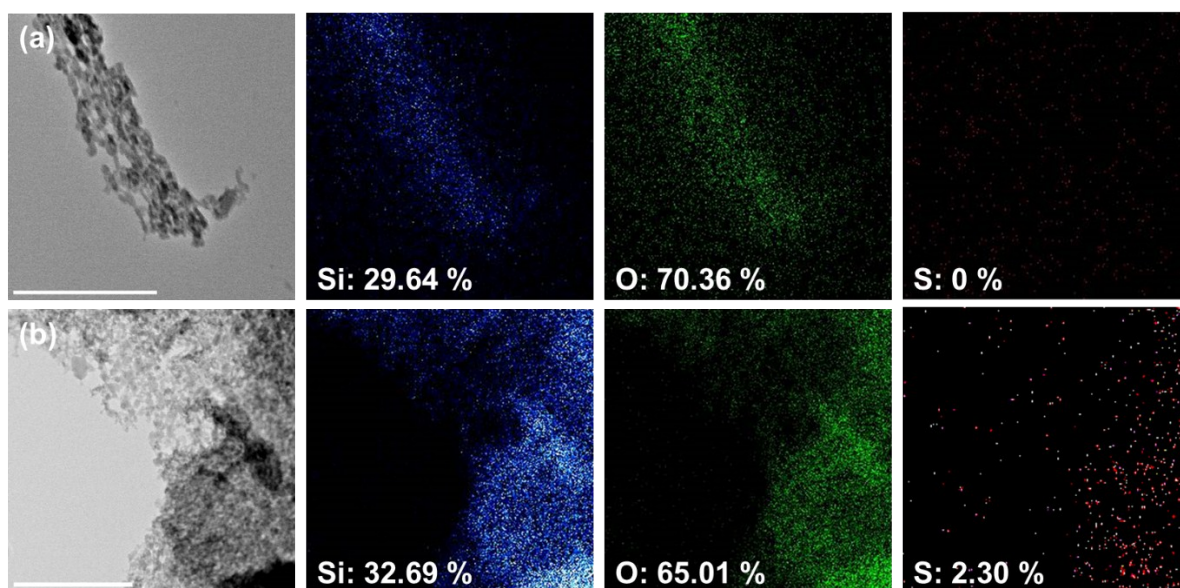


Fig. S4. TEM-EDS mapping image of (a) SiO₂-R and (b) thiol-SiO₂ catalysts (scale bars are 300 nm).

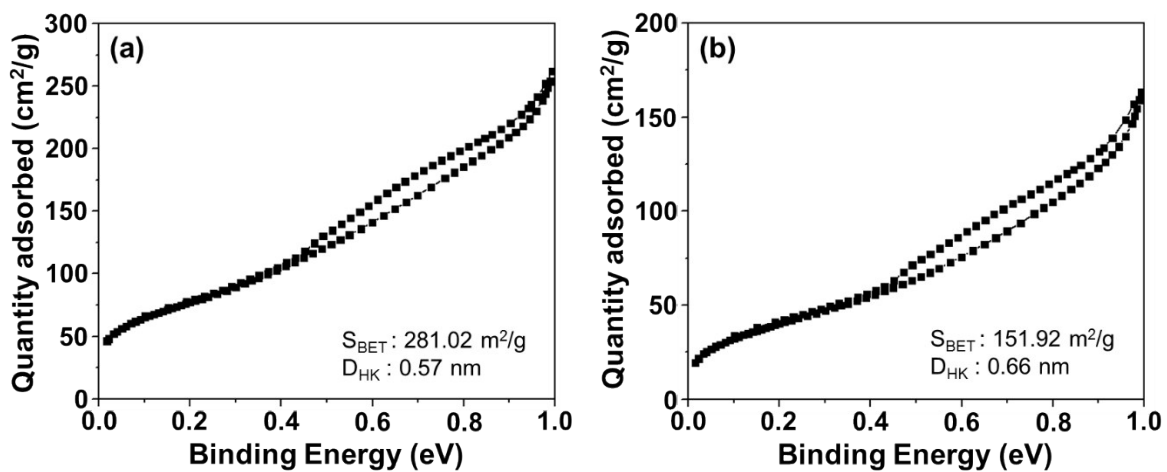


Fig. S5. BET hysteresis loop during N₂ adsorption/desorption with (a) rice husk derived SiO₂-R and (b) thiol-SiO₂.

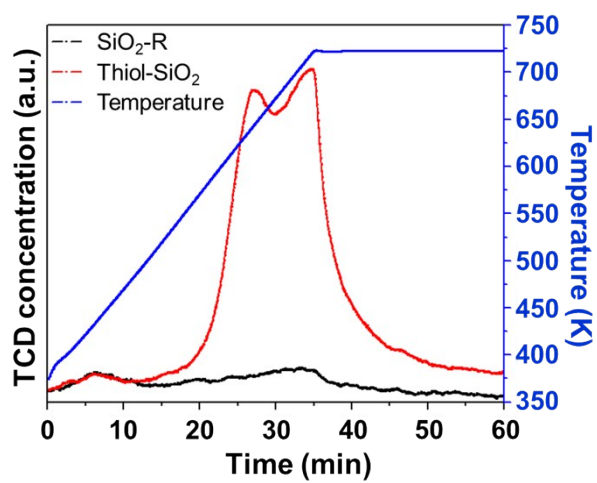


Fig. S6. NH₃-TPD curves of rice husk derived SiO₂-R and thiol-SiO₂.

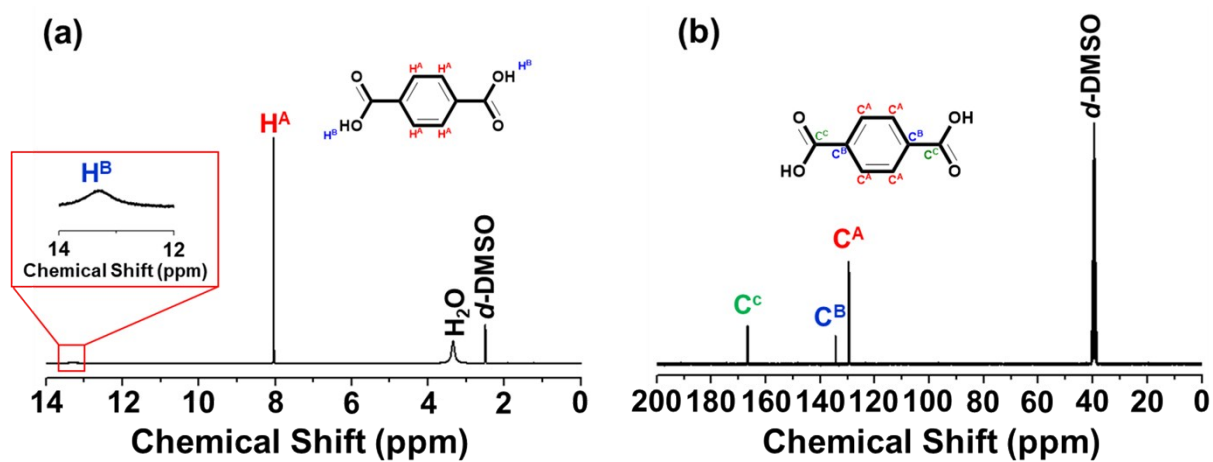


Fig. S7. (a) ¹H NMR and (b) ¹³C NMR of terephthalic acid (TPA) from PET hydrolysis

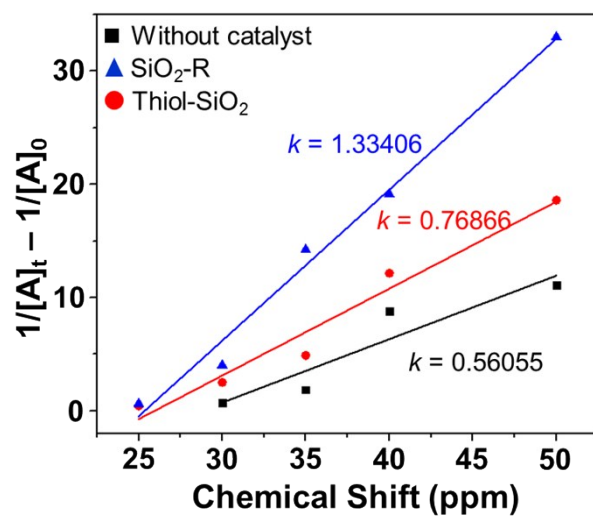


Fig. S8. Reaction constant (k) calculation of PET hydrolysis by without catalyst, rice husk derived SiO₂-R and thiol-SiO₂.

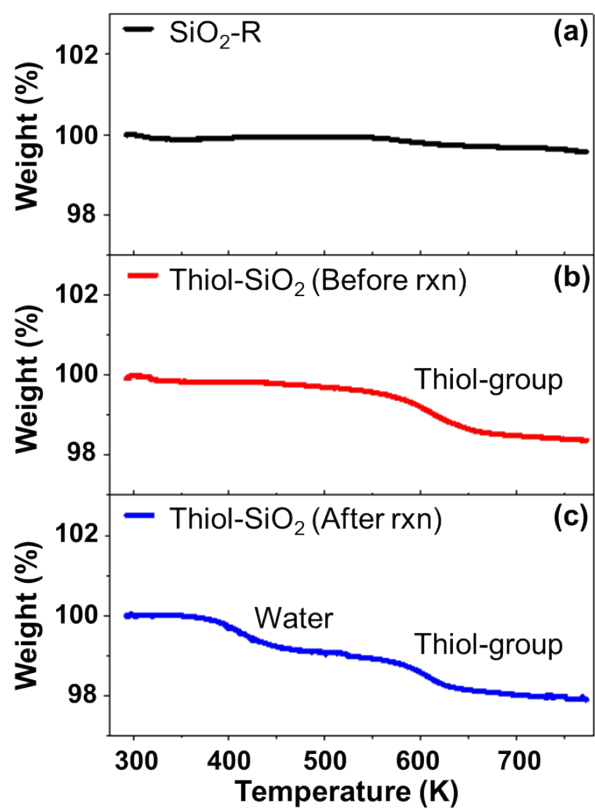


Fig. S9. Thermal gravity analysis curves of (a) SiO₂-R catalyst and Thiol-SiO₂ catalyst (b) before reaction and (c) after 6 cycles of reaction.

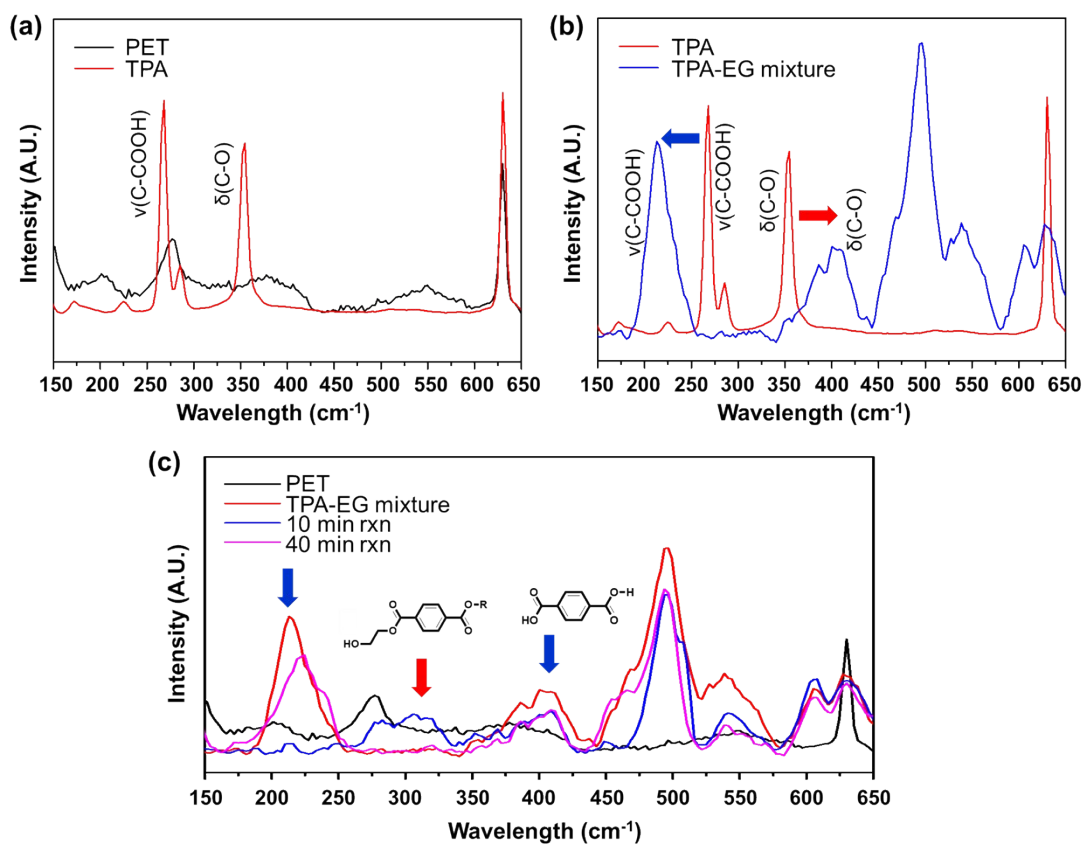


Fig. S10. Raman spectra of (a) PET and TPA, (b) TPA and TPA-EG mixture in water and (c) 10 min and 40 min reacted solution.

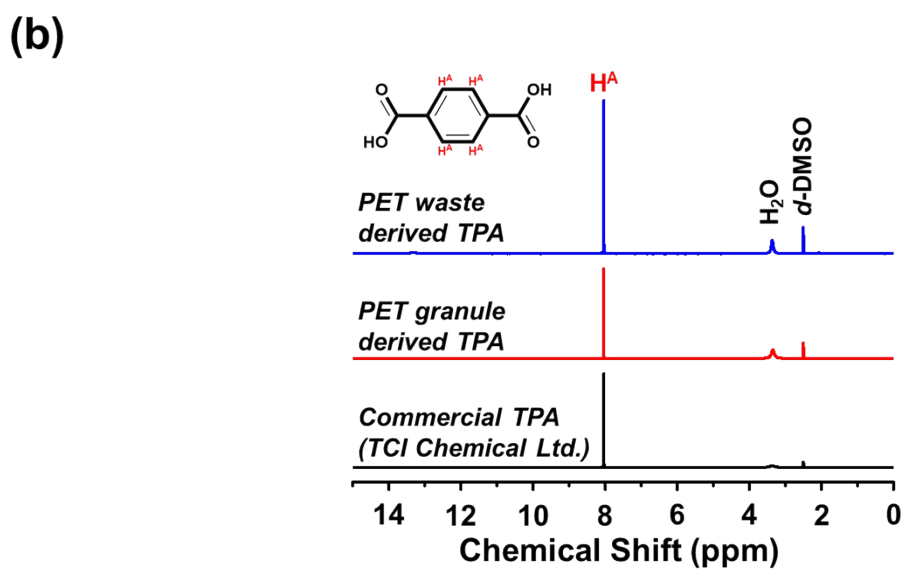
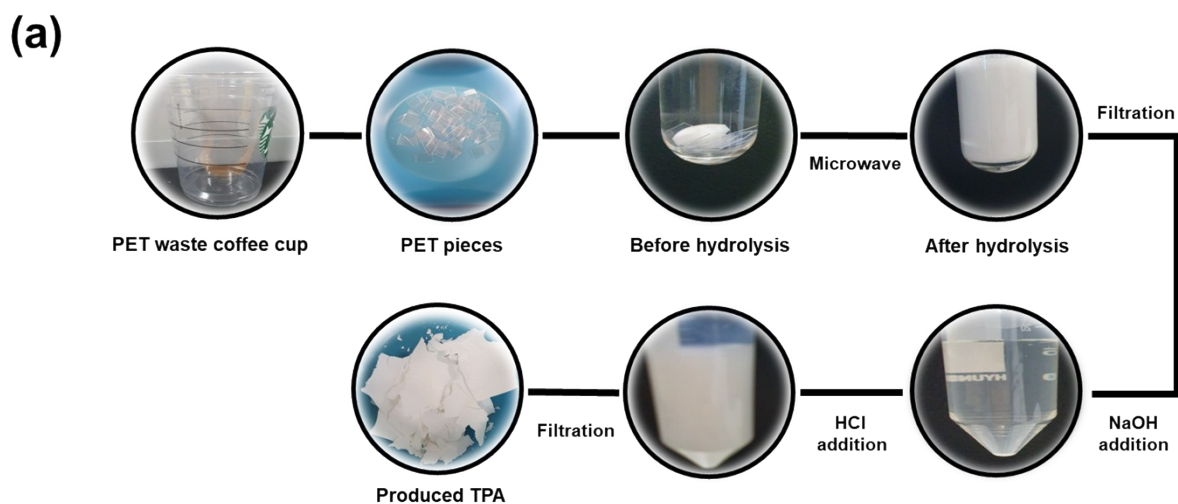


Fig. S11. (a) images of producing TPA from PET waste by microwave-assisted hydrolysis, (b) ¹H NMR of PET waste derived TPA, Aldrich PET granule derived TPA and reagent grade TPA purchased from TCI chemical Ltd.

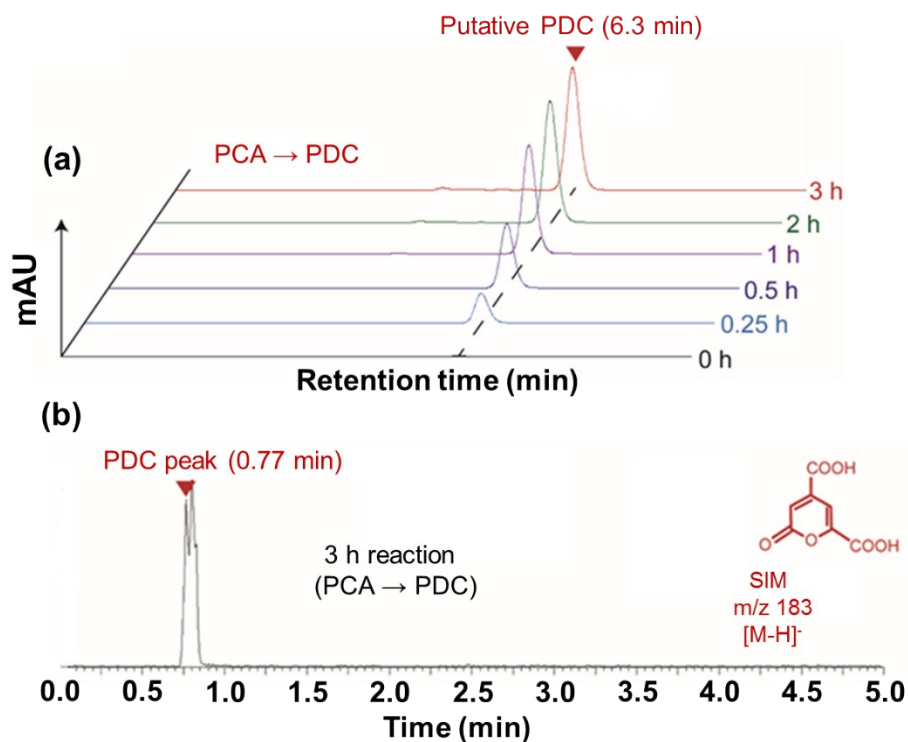


Fig. S12. Identification of PDC synthesis by whole cell conversion of PCA to PDC. (a) Time course HPLC chromatogram, (b) Putative PDC peak was increased by whole cell conversion reaction using *E. coli* PDC_{PCA} ($OD_{600}=30$) as reaction time was increased confirmation of PDC synthesis by LC-MS (SIM mode).

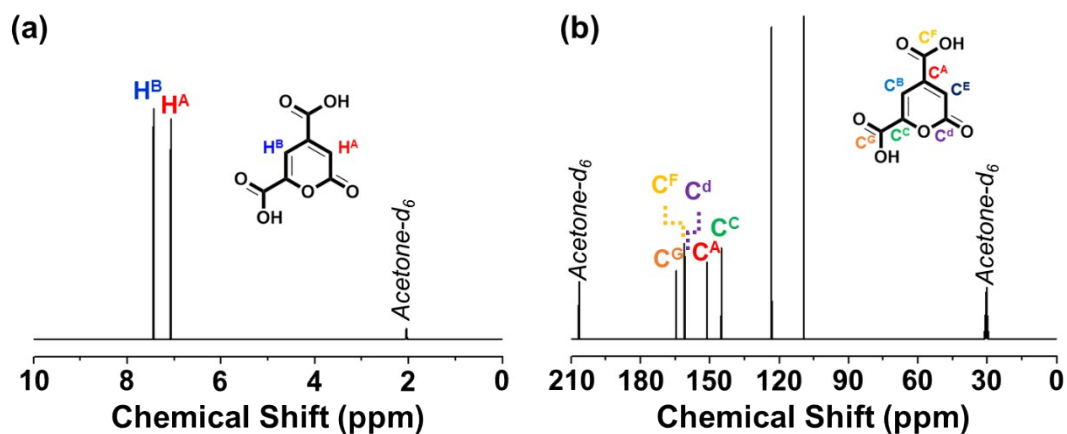


Fig. S13. (a) ^1H NMR and (b) ^{13}C NMR spectra of purified PDC.

Purified PDC through microbial conversion is confirmed by NMR spectroscopy. In ^1H proton NMR shown in Fig. S12 (a), two peaks appeared due to two kinds of protons in pyran ring of PDC (denoted as H^{A} and H^{B}), which has chemical shift value of 7.06 ppm and 7.43 ppm, respectively. The ^{13}C carbon NMR spectrum of PDC is also shown in Fig. S12 (b). PDC have seven kinds of carbons, which five are in pyran ring structure, shows chemical shift value of 151.23 ppm (denoted as C^{A}), 109.45 ppm (denoted as C^{B}), 145.10 ppm (denoted as C^{C}), 160.82 ppm (denoted as C^{D}) and 123.37 ppm (denoted as C^{E}). The other two types of carbons for each carboxylic acid groups in PDC but differences in distance with oxygen atoms in pyran ring, which are 161.17 ppm (denoted as C^{F}) and 164.61 ppm (denoted as C^{G}). Above ^1H and ^{13}C NMR results of synthesized PDC are in a good accordance with previously reported NMR spectra of PDC.¹

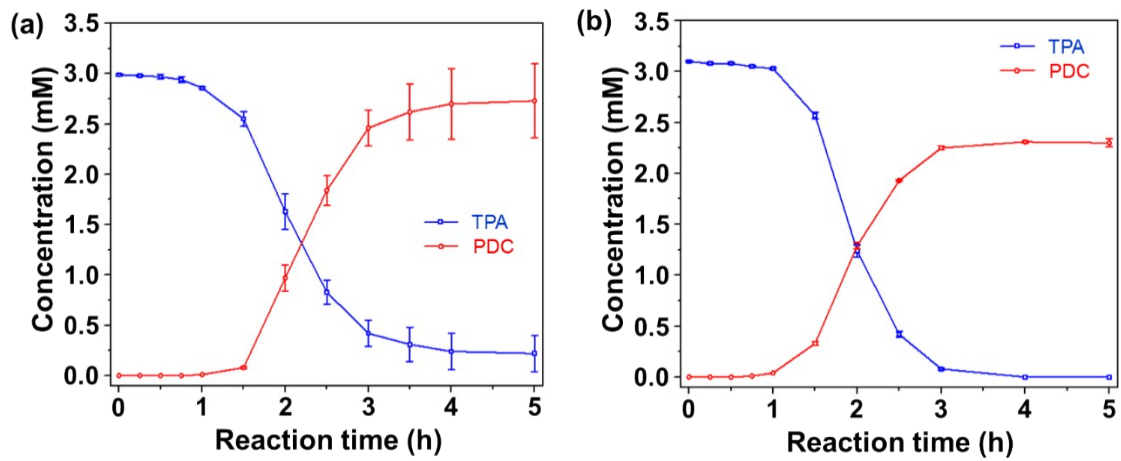


Fig. S14. Time course profiling of the TPA consumption and PDC production in (a) the single-catalyst system comprised with *E. coli* PDC_{TPA} strain (OD₆₀₀=30) and (b) The double-catalyst system comprised with *E. coli* PCA_{strain} (OD₆₀₀=10) and *E. coli* PDC_{PCA} strain (OD₆₀₀= 30).

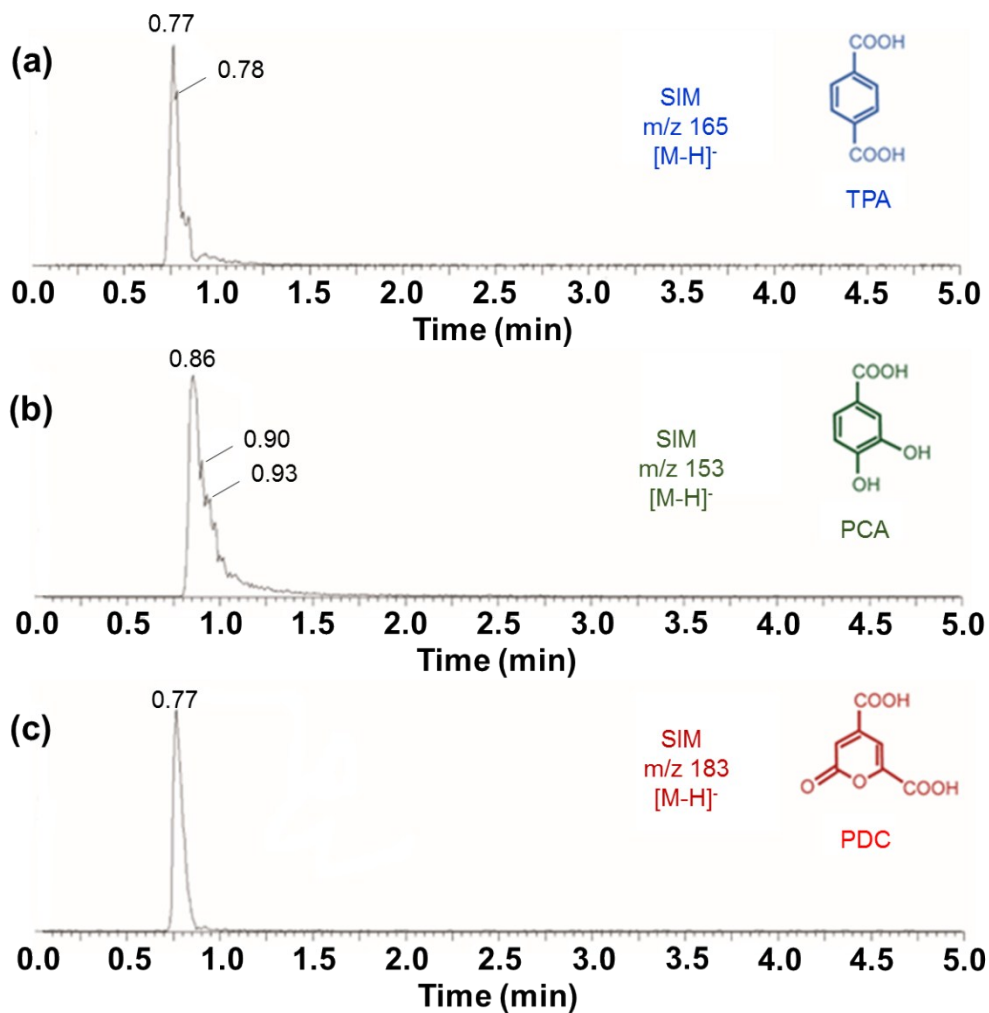


Fig. S15. LC-MS SIM chromatograms of (a) TPA standard, (b) PCA standard and (c) PDC authentic standard obtained by whole cell conversion reaction of PCA.

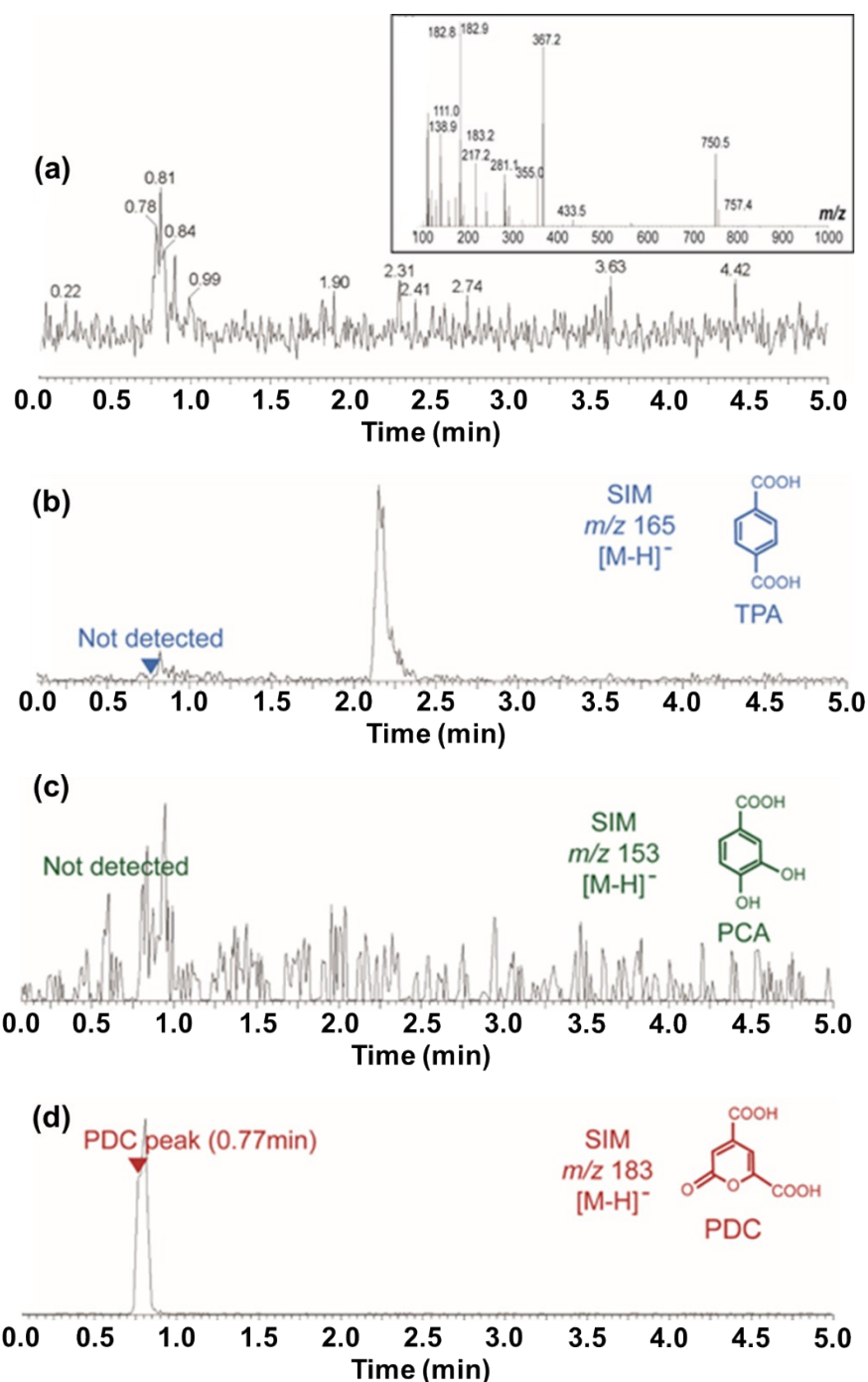


Fig. S16. LC-MS analysis of reaction mixture. (a) LC-MS scan mode of reaction mixture. LC-MS SIM mode to identify (b) TPA, (c) PCA and (d) PDC in reaction mixture. Reaction mixture was obtained by whole cell conversion reaction with TPA and double-catalyst system comprised with *E. coli* PCA strain (OD₆₀₀=15) and *E. coli* PDCPCA strain (OD₆₀₀= 35) for 3 h at 300 K and 200 rpm in TG buffer system (pH 7.0).

REFERENCES

1. J. M. Perez, W. S. Kontur, M. Alherech, J. Coplien, S. D. Karlen, S. S. Stahl, T. J. Donohue and D. R. Noguera, *Green Chem.* 2019, **21**, 1340-1350.