Supplementary Materials for

Spatial Niche Construction for Consortium-based Consolidated Bioprocessing System

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Supplementary Text

Strain and Media

S. cerevisiae BY4741 was cultivated in medium containing (in g liter^{-1}): KH_2PO_4, 3; $(NH_4)_2SO_4$, 5; $MgSO_4 \cdot 7H_2O$, 0.5; uracil, 0.15; and trace element stock, 1 mL liter^{-1}. The trace element stock containing (in g liter^{-1}): EDTA, 15; $ZnSO_4 \cdot 7H_2O$, 4.5; $CoCl_2 \cdot 6H_2O$, 0.3; $MnCl_2 \cdot 4H_2O$, 1; $CuSO_4 \cdot 5H_2O$, 0.3; $CaCl_2 \cdot 2H_2O$, 4.5; $FeSO_4 \cdot 7H_2O$, 3; $NaMoO_4 \cdot 2H_2O$, 0.4; H_3BO_3, 1; KI, 0.1; and glucose, 40. And the filter-sterilized vitamin solution was added medium (10 mL) in 50 mL serum bottle sterilized by high temperature steam. The final vitamin containing (in mg liter^{-1}): biotin, 0.05; calcium pantothenate, 1; nicotinic, 1; inositol, 25; thiamine HCl, 1; pyridoxine HCl, 1 and para-aminobenzoic acid, 0.2.

In the coculturing process, *T. asperellum* and *S. cerevisiae* BY4741 were cultivated in medium containing (in g liter^{-1}): microcrystalline cellulose, 40; urea, 0.3; peptone, 0.75; $(NH_4)_2SO_4$, 1.4; KH_2PO_4, 2; $MgSO_4 \cdot 7H_2O$, 0.3; yeast extract, 0.25; $CaCl_2$, 0.35; and trace element stock, 1 mL liter^{-1}. The trace element stock containing (in g liter^{-1}): $FeSO_4 \cdot 7H_2O$, 5; $MnSO_4 \cdot 4H_2O$, 1.6; $ZnSO_4 \cdot 7H_2O$, 1.4; and $CoCl_2 \cdot 6H_2O$, 20. And the filter-sterilized vitamin solution was added medium (10 mL) in 50 mL serum bottle sterilized by high temperature steam. The final vitamin containing (in mg liter^{-1}): biotin, 0.05; calcium pantothenate, 1; nicotinic, 1; inositol, 25; thiamine HCl, 1; pyridoxine HCl, 1 and para-aminobenzoic acid, 0.2.

L. paracasei was cultivated in medium containing (in g liter^{-1}): peptone, 10; beef extract, 0.75; yeast extract, 4; glucose, 20; tween 80, 0.3; sodium acetate, 5; ammonium citrate, 2; K_3PO_4, 2; $MnSO_4$, 0.05; and $MgSO_4$, 0.2.

In the coculturing process, *T. asperellum* and *L. paracasei* were cultivated in medium containing (in g liter^{-1}): microcrystalline cellulose, 40; urea, 0.3; peptone, 0.75; $(NH_4)_2SO_4$, 1.4; KH_2PO_4, 2; $MgSO_4 \cdot 7H_2O$, 0.3; yeast extract, 0.25; $CaCl_2$, 0.35; and trace element stock, 1 mL liter^{-1}. The trace element stock containing (in g liter^{-1}): $FeSO_4 \cdot 7H_2O$, 5; $MnSO_4 \cdot 4H_2O$, 1.6; $ZnSO_4 \cdot 7H_2O$, 1.4; and $CoCl_2 \cdot 6H_2O$, 20.

Chlorella sp. GY-H4 was cultivated in BG-11medium containing (in g liter^{-1}): citric acid, 6.0; ferric ammonium citrate, 6.0; EDTA, 1; $NaNO_3$, 1.5; $K_2HPO_4 \cdot 2H_2O$, 0.051; $MgSO_4 \cdot 7H_2O$, 0.075; $CaCl_2$ 0.024; Na_2CO_3, 0.02; A5 trace mineral solution 1 mL liter^{-1}. The A5 trace mineral solution containing (in mg liter–1): H_3BO_4, 2.86; $MnCl_2 \cdot 4H_2O$, 1.81; $ZnSO_4 \cdot 7H_2O$, 0.222; $Na_2MoO_4 \cdot 2H_2O$, 0.391; $CuSO_4 \cdot 5H_2O$, 0.079; and $Co(NO_3)_2 \cdot 6H_2O$, 0.049.

Construction of *T. asperellum* and *L. paracasei*

For the CBP consortium, *T. asperellum* were cultivated was first inoculated under aerobic conditions in 3D printed bioreactor for 48 hours. Then, the hydrogel-based living materials containing *L. paracasei* were then inoculated. During the fermentation process, concentrations of lactic in the sample was determined. Each experiment was performed in triplicates.

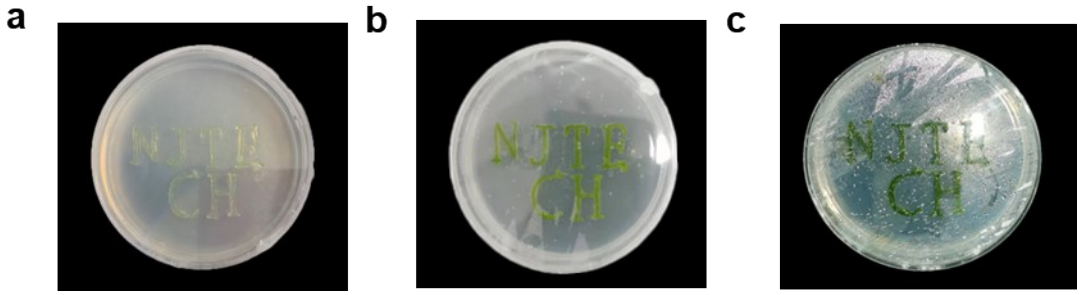

Figure S1. Biocompatibility characterization of living materials containing *Chlorella* sp. GY-H4. (a-c) Photographs of living materials containing *Chlorella* sp. GY-H4 printed directly onto agar tablet on the first, third and seventh days, respectively.

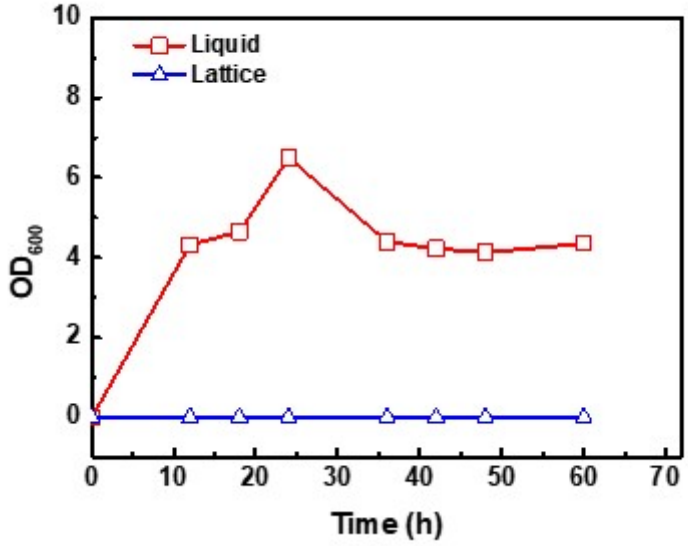

Figure S2. Comparison of OD_{600} curves with liquid culture and yeast-laden lattice hydrogel-based living materials.

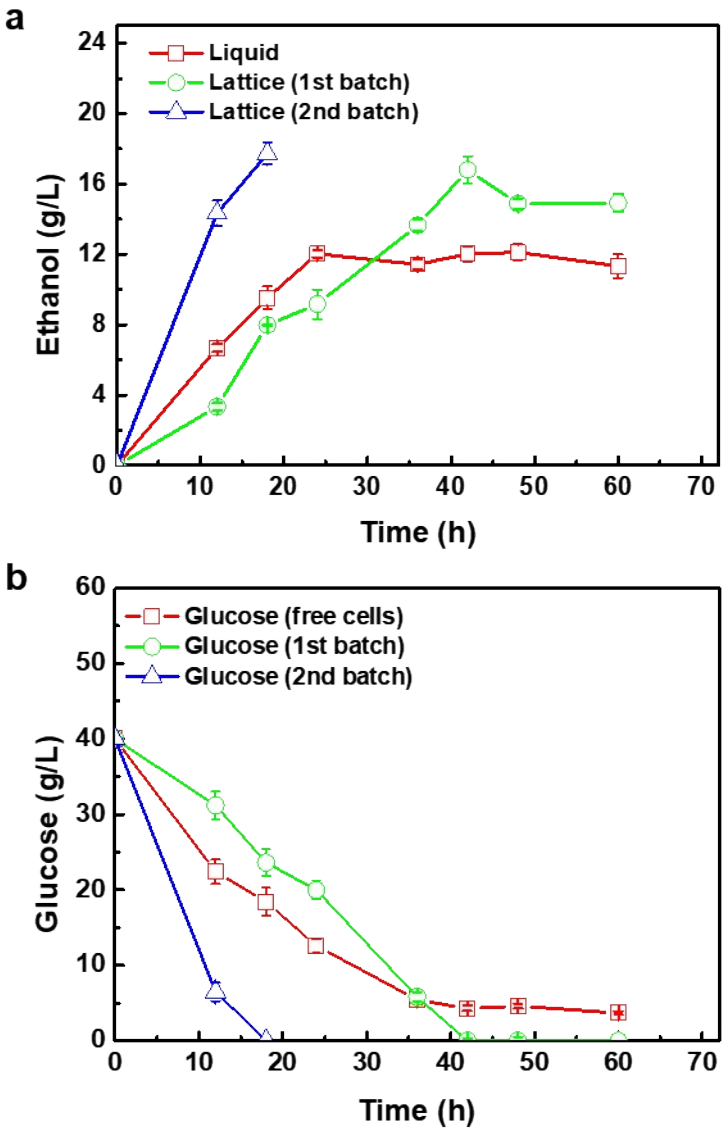

Figure S3. The performance of ethanol production and glucose consumption by using liquid culture, first batch and second batch of living materials. (a) Comparison of ethanol titer with by using liquid culture, first batch and second batch of living materials, (b) Comparison of glucose consumption by using liquid culture, first batch and second batch of living materials.

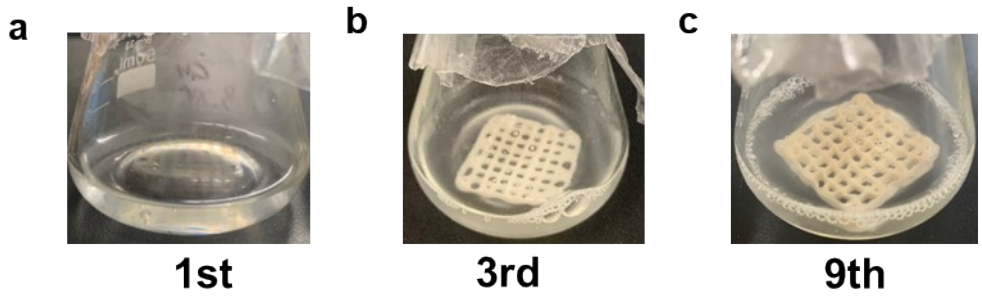

Figure S4. Photographs of living materials after 1st, 3rd ,9th batch fermentation.

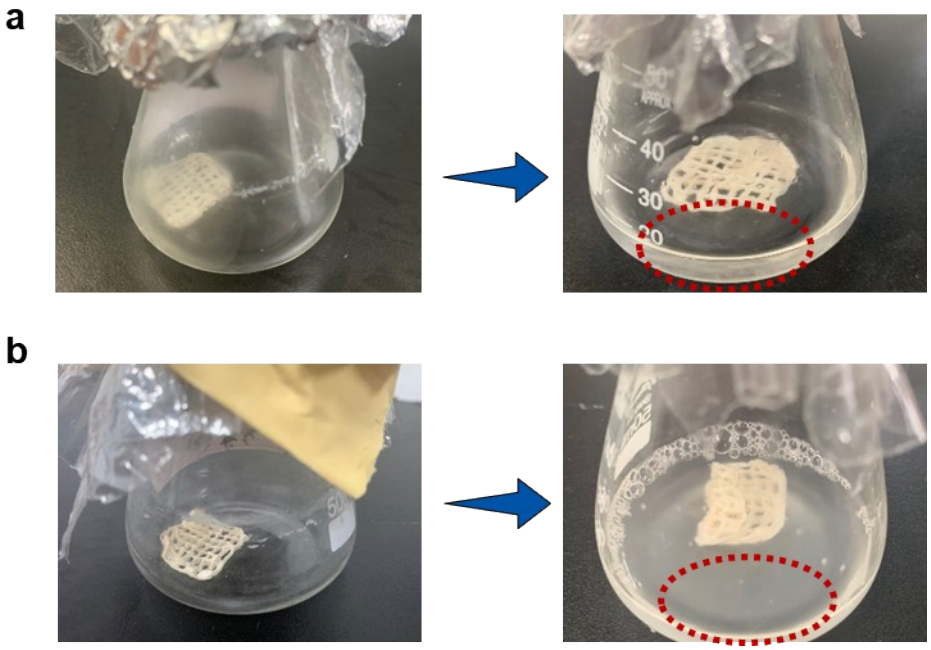

Figure S5. Preservation methods of living materials containing *S. cerevisiae* BY4741 under different conditions. (a) Photographs of living materials preserved by cold stage at 4℃, (b) Photographs of living materials preserved by lyophilization.

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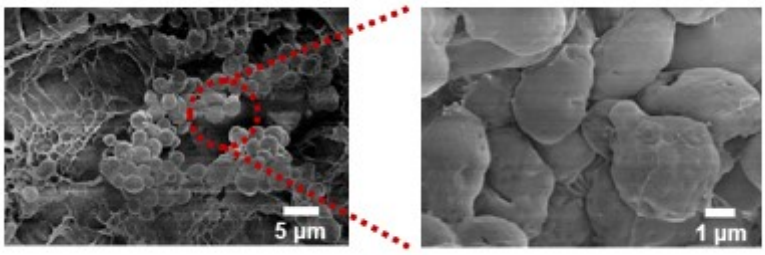

Figure S6. SEM analysis of living materials containing *S. cerevisia*e BY4741 after fermentation.

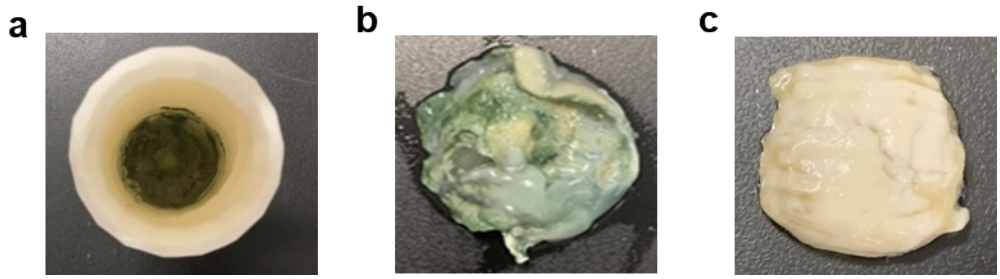

Figure S7. Photographs of biofilm of *T. asperellum*. (a, b) Photographs of biofilm of *T. asperellum* on 3D printed device surface, (c) Photograph of biofilm of *T. asperellum* on living materials surface.