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Supplementary Information

Reconfiguring workup steps in multi-cycle extractive bioconversion for sustainable fatty alcohol production: a process engineering approach

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Content **Section**

S1. Detailed Experimental Procedures

- **Extractive bioconversion and testing of different configurations and reconfigurations**

In all following experiments, the cells collected from the previous step were resuspended with the prepared 100 mM potassium phosphate buffer (referred as "buffer"). They were adjusted to OD_{600} of 30. A certain amount of glucose was added into the buffer. 10 mL of the buffer was aliquoted into a 100 mL bottle. Dodecane was added into some bottles to perform the extractive bioconversion. The experiment without *in situ* dodecane layering was noted as "control." Overall, the experiments were divided into three sets:

1st Set of Experiment: Study and optimization of the in-situ extraction for the bioconversion to produce fatty alcohols

The first set of experiments aimed to study the effects of dodecane layering and to determine an optimal dodecane-to-buffer ratio. Four bottles containing 10 mL of the buffer (with cells and 50 mM glucose added) were arranged. The 1st bottle was the "control," without the dodecane layer. The 2nd, 3rd, and 4th bottles were added with 0.5, 1, and 2 mL of dodecane. All the bottles were incubated with 250 rpm shaking and at 25 °C for 48 h. At the end of 48 h, the bottle was brought to settle for clear separation between top and bottom layers. Both layers were analyzed to determine the fatty alcohol contents. The dodecane-to-buffer ratio that gave the highest fatty alcohol production was considered as optimal, and used in subsequent experiments. Each experiment was done in triplicate.

2nd Set of Experiment: Multi-cycle extractive bioconversion with different configurations (C0, C1, C2, C3, and C4) between cycles

The second set of experiments aimed to study fatty alcohol production using different configurations. The experiment was done in 4 cycles with 6 h/cycle. There were three workup steps that could be implemented during the transition between cycles (e.g. 1st cycle to 2nd cycle). The three workup steps were glucose (substrate) addition, dodecane change, and buffer change. The dodecane and buffer exchanges meant the replacement of the existing dodecane and buffer phases with fresh dodecane and buffer, respectively. The buffer exchanges unavoidably required the addition of glucose, as the glucose in the existing buffer phase was discarded. The same total amount of glucose was used in all experiments: 0.4 mmol or 72 mg.

There were five different experiments tested: control, C0, C1, C2, C3, and C4:

- In the control, no dodecane layer was added. No workup steps were implemented at any transition between cycles.
- In **C0**, no workup steps were implemented during the transition between cycles.
- In **C1**, glucose was added before each subsequent cycle. Therefore, in this configuration, the initial glucose amount (at $t = 0$) was not 72 mg. In contrast, 18 mg of glucose was added at the beginning of each cycle.
- In **C2**, the existing dodecane was replaced with fresh dodecane before each subsequent cycle. After the bioconversion step in each cycle, the bottle was first transferred to a centrifuge tube, and centrifuged at 5000 rpm at 4 for 10 min to separate the immiscible

aqueous and solvent phases. The entire top (dodecane) layer was withdrawn from the tube. 1 mL of dodecane was then added to the remaining bottom (aqueous, buffer) layer before performing the next bioconversion cycle.

- In **C3**, the buffer phase was replaced with a fresh buffer before each cycle. After the bioconversion step at each cycle, the bottle was first transferred to a centrifuge tube, and centrifuged at 5000 rpm at 4 for 10 min for immiscible liquid-liquid separation. The entire top (dodecane) layer was removed from the tube and kept in another tube. The supernatant (mainly the buffer) was then removed while the cell pellet was collected. Fresh buffer (10 mL) was added to resuspend the cell pellet. Glucose (18 mg) was added to the new buffer phase. The collected dodecane from the previous cycle was then added back to the buffer phase before performing the next bioconversion cycle.
- In **C4**, all the workup steps (i.e., glucose addition, dodecane change, and buffer change) were implemented. After the bioconversion step at each cycle, the bottle was first transferred to a centrifuge tube, and centrifuged at 5000 rpm at 4 for 10 min for immiscible liquid-liquid separation. The entire top (dodecane) layer was withdrawn from the tube. The supernatant (mainly the buffer) was then removed while the cell pellet was collected. Fresh buffer (10 mL) was added to resuspend the cell pellet. Glucose (18 mg) was added to the new buffer phase. 1 mL of dodecane was then added to the buffer phase before performing the next bioconversion cycle.

A bioconversion step with a period of 6-hr was performed in each cycle. At the end of each cycle, the dodecane phase was sampled for analysis of the fatty alcohol content. In this set of experiments, the samples were also analyzed for the acetate level. Each experiment was done in triplicates.

3rd Set of Experiment: Reconfiguration

This set of experiments aimed to extend the experiment from 4 cycles to 8 cycles and rearrange (so-called "reconfigure") the different configurations (C2, C4) at different transitions between cycles.

There were six different experiments tested: control, C2, C3, R1, R2, and R3:

- In the control, no dodecane layer was added. No workup steps were implemented at any transition between cycles.
- In **C2**, the existing dodecane was replaced with fresh dodecane before each subsequent cycle. After the bioconversion step in each cycle, the bottle was first transferred to a centrifuge tube, and centrifuged at 5000 rpm at 4 ^{o}C for 10 min for immiscible liquid-liquid separation. The entire top (dodecane) layer was withdrawn from the tube. 1 mL of dodecane was then added to the remaining bottom (aqueous, buffer) layer before performing the next bioconversion cycle. These steps were done for all the transitions between cycles (from $(i-1)^{th}$ cycle to i^{th} cycle). To keep the same total amount of glucose as the other experiments, 144 mg of glucose was added initially at the beginning of the 1st cycle.
- In **R1**, we used the same procedure as C2. However, instead of changing dodecane at every transition, we conducted the dodecane change at every second transition.
- In **R2**, we used the same procedure as C2. However, instead of changing dodecane at every transition, we conducted the dodecane change at every third transition.

- In **R3**, we used the same procedure as C2. However, instead of changing dodecane at every transition, we conducted the dodecane change at every forth transition.
- In **R4 (0.8 mmol total glucose)**, the workup steps were performed for only two transitions. At the transition from the 1st to 2nd cycles, the steps of C2 were performed. At the transition from 4th to 5th cycles, the steps of C4 were performed. 72 mg of glucose was added initially at the beginning of the 1st cycle, and 72 mg of glucose was added at the beginning of the 5th cycle.
- In **R4 (1.6 mmol total glucose)**, the workup steps were performed for only two transitions. At the transition from the 1st to 2nd cycles, the steps of C2 were performed. At the transition from 4th to 5th cycles, the steps of C4 were performed. 144 mg of glucose was added initially at the beginning of the 1st cycle, and 144 mg of glucose was added at the beginning of the 5th cycle.
- In **R4 (3 mmol total glucose)**, the workup steps were performed for only two transitions. At the transition from the 1st to 2nd cycles, the steps of C2 were performed. At the transition from 4th to 5th cycles, the steps of C4 were performed. 270 mg of glucose was added initially at the beginning of the 1st cycle, and 270 mg of glucose was added at the beginning of the 5th cycle.
- In **R4 (4 mmol total glucose)**, the workup steps were performed for only two transitions. At the transition from the 1st to 2nd cycles, the steps of C2 were performed. At the transition from 4th to 5th cycles, the steps of C4 were performed. 360 mg of glucose was added initially at the beginning of the 1st cycle, and 360 mg of glucose was added at the beginning of the 5th cycle.
- In **R5**, the workup steps were performed for only two transitions. At the transition from the 1st to 2nd cycles, the steps of C2 were performed. At the transition from 4th to 5th cycles, the steps of C4 were performed. We distributed the glucose addition (total of 4 mmol or 720 mg) over the course of the bioconversion. At each cycle, we added 90 mg of glucose.

A bioconversion period of 6-hr was performed in each cycle. At the end of each cycle, the dodecane phase was sampled for analysis of the fatty alcohol content. Each experiment was done in triplicate.

S2. Detailed conditions for different experiments

Table S1. Detailed activities in workup steps in the second set of experiments*

Experiment	Workup step at the transition between cycles			
	0 → 1	1 → 2	2 → 3	3 → 4
Control*	72 mg glucose			
C0	72 mg glucose			
C1	18 mg glucose	18 mg glucose	18 mg glucose	18 mg glucose
C2	72 mg glucose			
C3	18 mg			
		18 mg glucose	18 mg glucose	18 mg glucose
C4	18 mg glucose			
		18 mg glucose	18 mg glucose	18 mg glucose

Table S2. Detailed activities in workup steps in the third set of experiments*

Experiment	Workup step at the transition between cycles							
	0 → 1	1 → 2	2 → 3	3 → 4	4 → 5	5 → 6	6 → 7	7 → 8
Control	144 mg glucose							
C2	144 mg glucose							
R1	144 mg glucose							
R2	144 mg glucose							
R3	144 mg glucose							
R4 (0.8 mmol)	72 mg glucose				72 mg glucose			
R4 (1.6 mmol)	144 mg glucose				144 mg glucose			
R4 (3 mmol)	270 mg glucose				270 mg glucose			
R4 (4 mmol)	360 mg glucose				360 mg glucose			
R5	90 mg glucose	90 mg glucose	90 mg glucose	90 mg glucose	90 mg glucose	90 mg glucose	90 mg glucose	90 mg glucose

*Explanation for each color box

Preparation step:		1. Resuspend cells and adjust OD_{600} to 30 with 10 mL buffer 2. Add glucose (amount as indicated in the table) 3. Add dodecane 1:10 ratio

Glucose addition:		1. Add glucose (amount as indicated in the table)
Dodecane change:		1. Centrifuge at 5000 rpm at 4°C for 10 min for liquid-liquid separation 2. Remove the dodecane phase (top phase) 3. Resuspend cells in a buffer then transfer solution into a new bottle and add dodecane at a volumetric ratio of 1:10 (dodecane-to-buffer)
Buffer change:		1. Exchange buffer after withdrawing dodecane 2. Resuspend cells and buffer and then transfer solution into bottle
Without workup steps		No activity

S3. Calculation of different parameters

A number of different parameters were estimated in this work. Their definitions are as follows:

- **Percent extraction (% Extraction) calculation**

This parameter is to determine the percentage of the mass of fatty alcohols being extracted to the dodecane (top) phase during the bioconversion experiment.

$$\%\ Extraction = \frac{mass\ of\ fatty\ alcohols\ in\ dodecane\ phase\ (extracted)}{total\ mass\ of\ fatty\ alcohols\ produced}$$

$$\%\ Extraction = \frac{\left(\sum_{i=1}^{4} C_{i,\,dodecane}\right) \cdot V_{dodecane}}{\left(\sum_{i=1}^{4} C_{i,\,dodecane}\right) \cdot V_{dodecane} + \left(\sum_{i=1}^{4} C_{i,\,buffer}\right) \cdot V_{buffer}}$$

Where $C_{i,\,dodecane}$ is the concentration of a specific fatty alcohol compound ($i =$ oleyl alcohol, cetyl alcohol, palmitoleyl alcohol, and myristyl alcohol) in the dodecane phase, $C_{i,\,buffer}$ is the concentration of a specific fatty alcohol compound ($i =$ oleyl alcohol, cetyl alcohol, palmitoleyl alcohol, and myristyl alcohol) in the buffer phase, $V_{dodecane}$ and V_{buffer} are the volumes of the dodecane and buffer phases, respectively.

- **E-factor and complete E-factor (cE-factor) calculation**

E-factor is a sustainability metric, representing the ratio of the amount of waste generated per the amount of key products produced. The lower the value, the more sustainable the process. E-factor does not take into account the mass of water. However, in order to obtain a comprehensive understanding of process sustainability, we also calculated the complete E-factor (cE-factor), which includes water. Here, the E-factor and cE-factor values were calculated for only the bioconversion. The waste generated during the cell preparation and downstream processes (i.e., purification of products) were not included. Our experiments and most of the works in this report showed almost complete conversion of the substrates, so the mass of the substrate was negligible, and not included in the calculation. The mass of the biomass (i.e., cells) was also not included.

$E-factor = \frac{\sum Mass\ of\ waste\ (g)\ (except\ water)}{Mass\ of\ product\ (g)}$	$E-factor = \frac{M_{solvent} + M_{buffer}}{M_{product}}$
$cE-factor = \frac{\sum Mass\ of\ waste\ (g)}{Mass\ of\ product\ (g)}$	$cE-factor = \frac{M_{solvent} + M_{buffer} + M_{water}}{M_{product}}$

Where $M_{solvent}$, M_{buffer}, and M_{water} are the masses of the solvent used for layering or final extraction (e.g., dodecane, ethyl acetate), the buffer compounds (e.g., KH_2PO_4, K_2HPO_4), and water, respectively.

S4. Detailed Analytical Procedures

- **Analysis of fatty alcohol**

In the first set of experiments, both phases (dodecane and buffer) were sampled and analyzed for their fatty alcohol content. In the second and third sets of experiments, only the dodecane phase was sampled and analyzed. Samples from the buffer phase were extracted with ethyl acetate containing internal standards (10μM dodecane and 250 μM tetradecane) at a sample-to-ethyl acetate (volume) ratio of 1:1. The extraction was vigorously vortexed before centrifugation at 10,000 rpm and 4 °C for 10 min. The clear organic phase was taken for analysis by gas chromatography/mass spectrometry (GC/MS). Samples from the dodecane phase were directly diluted in ethyl acetate at a dilution factor of 100. The diluted samples were then analyzed by GC/MS. The GC/MS analysis used a HP-5MS column with helium as a carrier gas (flow rate of 7 ml/min). The injection temperature was 250 °C. The sample was split at a ratio of 10:1. The temperatures in the oven were programmed as follows: maintenance at 60 °C for 3 min; heating to 200 °C at a rate of 10 °C min^{-1}; maintenance at 200 °C for 2 min; heating to 260 °C at a rate of 20 °C min^{-1} for 3 min. The ions (*m/z*) used for the qualitative and quantitative analysis of oleyl alcohol, cetyl alcohol, palmitoleyl alcohol, and myristyl alcohol in GC/MS are shown in **Table S3**. Concentrations of the fatty alcohols were determined from the standard curve.

- **Analysis of acetate**

Samples from the buffer phase (1 mL) were centrifuged for 5 min at 10,000 rpm, 4 °C, and the supernatant (aqueous phase) was filtered through a 0.2-μm syringe filter. The filtered samples were then analyzed by HPLC–DAD-RI detection (Agilent) using a c-18 column (Water; Symmatry300) operated at 35 °C. The analytes were eluted with 30 mM sodium phosphate (Na_2HPO_4) containing 0.075% of phosphoric acid in H_2O at a flow rate of 0.3 ml/min. The detection wavelength was set at 210 nm. The concentrations of acetate were determined by the standard curve.

- **Analysis of glucose**

Samples from the buffer phase (1 mL) were centrifuged for 5 min at 10,000 rpm, 4 °C, and the supernatant (aqueous phase) was filtered through a 0.2-μm syringe filter. The filtered samples were then analyzed by HPLC–QTOF (Agilent), column Hi-plex Ca^{2+} (Duo), with a mobile phase of 0.01% of formic acid in H_2O: ACN (80:20) operated at 80 °C with a flowrate of 0.3 min/ml.

- **Measurement of cell viability**

Samples from the buffer phase in the second set of experiments were obtained at different time points throughout the bioconversion: 6, 12, 18 and 24 h. The samples were diluted in sterile water (standard 10-fold dilution). The diluted solutions were then dropped onto LB agar plates. The

plates were incubated at 37 °C for 12 h. Colonies grown were counted and used for calculation of colony forming units (CFUs).

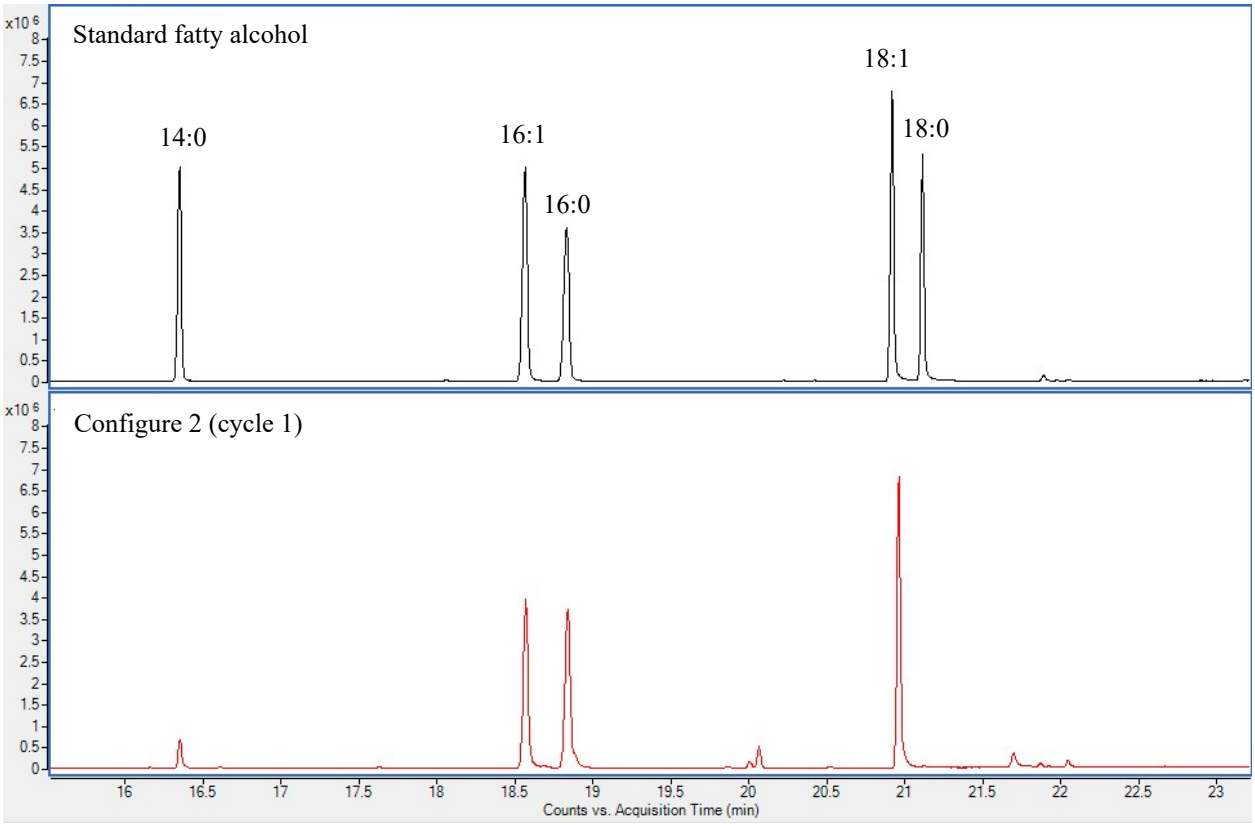


Figure S1. Analysis of fatty alcohols. (Top) GC-MS chromatograms of the standard fatty alcohols: myristyl alcohol (14:0), palmitoleyl alcohol (16:1), cetyl alcohol (16:0), oleyl alcohol (18:1) and stearyl alcohol (18:0) showing retention times of 16.35, 18.54, 18.94, 20.09 and 21.12 mins. (Bottom) The GC-MS chromatograms of the representative samples (C2 at the 1st cycle).

Table S3. The ions (*m/z*) for the qualitative and quantitative analysis of fatty alcohols

Compounds	Exact mass	Qualitative ion (molecular m/z)	Quantitative ion (most abundant m/z)
Myristyl alcohol (14:0)	214.23	213	83
Palmitoleyl alcohol (16:1)	240.25	239	55
Cetyl alcohol (16:0)	242.26	241	69
Oleyl alcohol (18:1)	268.28	268	82
Stearyl alcohol (18:0)	270.29	269	55

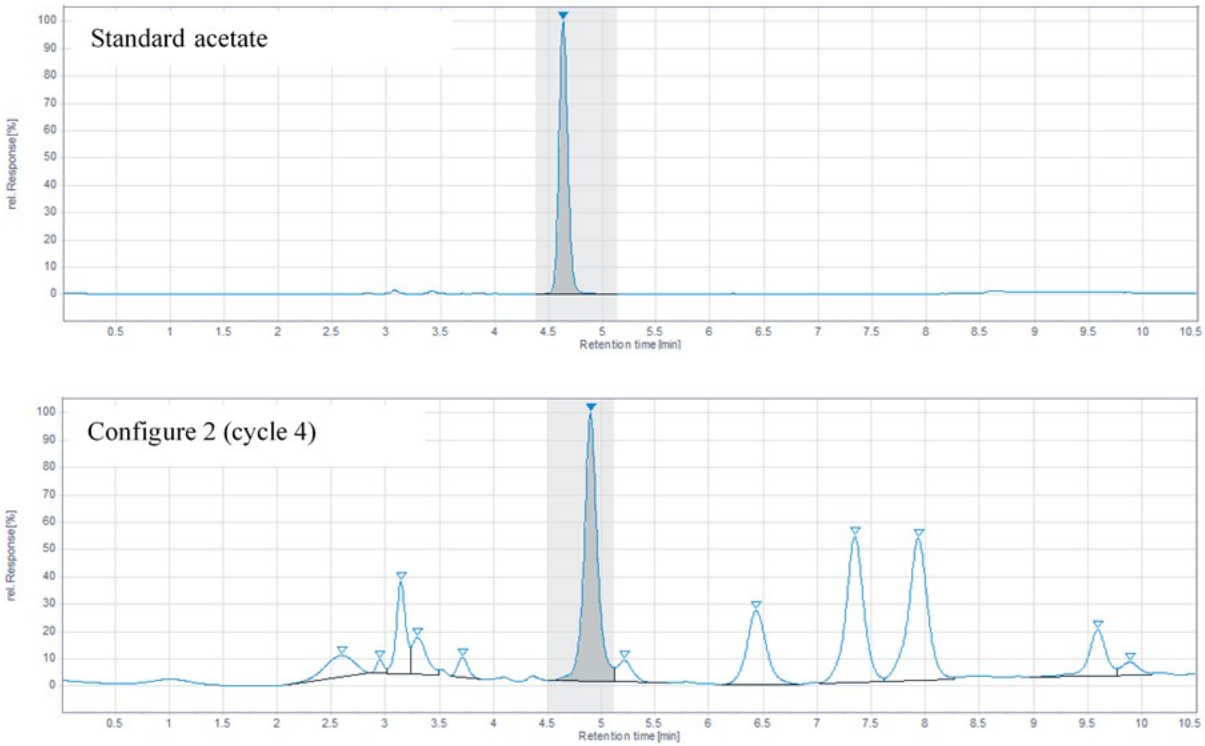
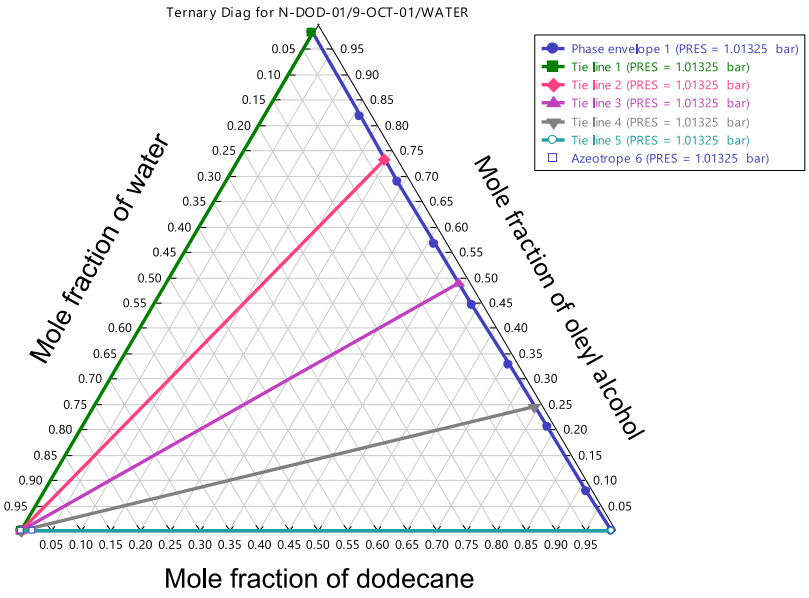


Figure S2. Analysis of acetate**.** (Top) The HPLC chromatogram of the standard acetate showing a retention time of 4.62 mins. (Bottom) The HPLC chromatogram of the acetate found in the representative sample (C2 at the 4th cycle).

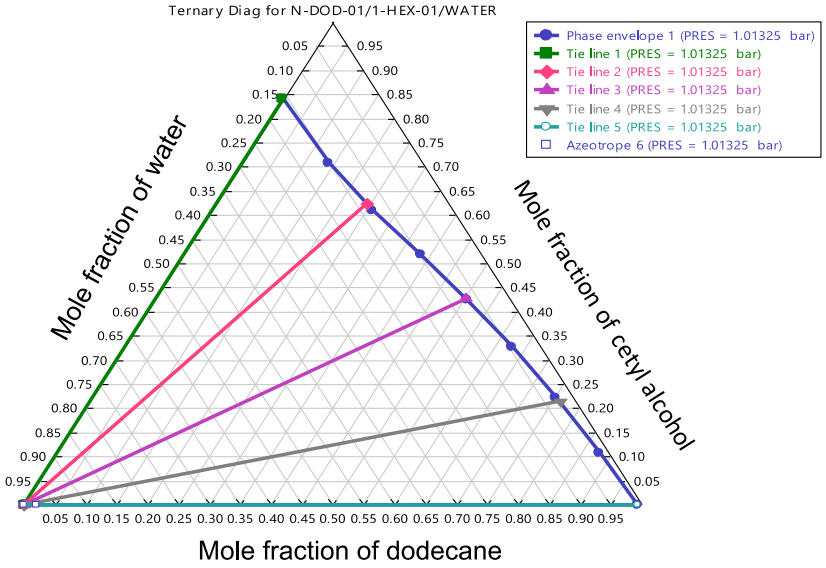
S5. Results of fatty alcohol saturation concentration and partition ratio

We also experimentally determined the fatty alcohol saturation concentration. This value represents a maximum amount of fatty alcohols that can be soluble in dodecane. In this test, we added cetyl alcohol, a representative fatty alcohol compound, into dodecane until the solution became turbid. We found that the saturation concentration in dodecane was 30 ± 5 mM. We also generated a ternary phase diagram for the equilibrium partition at 25 °C using a process simulation software (Aspen Plus). For the system of water-fatty alcohol-dodecane, a large two-phase region was observed as shown in Figure S3. Tie lines were drawn in this region. The two ends of each tie line represent the compositions (mole fractions) in the two immiscible phases. Most importantly, we observed that for all the fatty alcohols studied, the tie lines converged to the point in the left angle. At this point, the phase is rich with water, with very low compositions of dodecane and fatty alcohol. This clearly indicates that almost all fatty alcohols will prefer to partition into the dodecane phase.

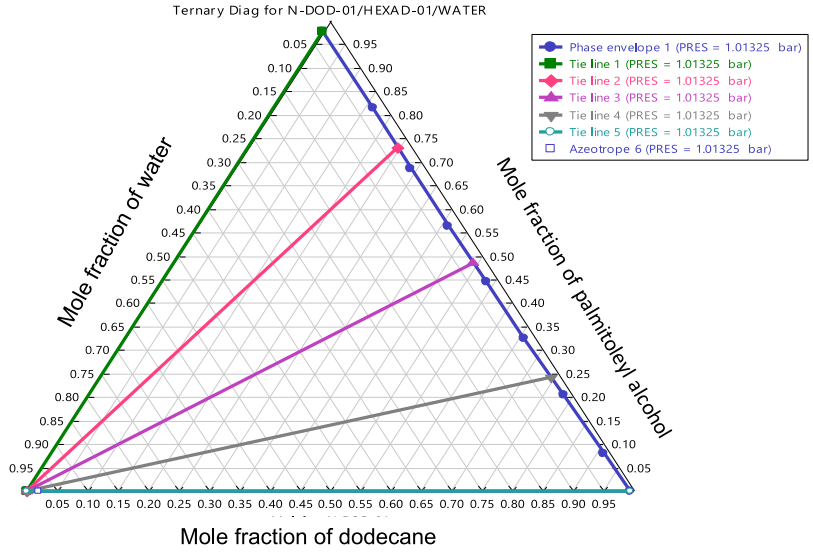
(a)



(b)


Ternary Diag for N-DOD-01/1-HEX-01/WATER
Phase envelope 1 (PRES = 1.01325 bar)
Tie line 1 (PRES = 1.01325 bar)
Tie line 2 (PRES = 1.01325 bar)
Tie line 3 (PRES = 1.01325 bar)
Tie line 4 (PRES = 1.01325 bar)
Tie line 5 (PRES = 1.01325 bar)
Azeotrope 6 (PRES = 1.01325 bar)
Mole fraction of water
Mole fraction of cetyl alcohol
Mole fraction of dodecane

(c)


Ternary Diag for N-DOD-01/HEXAD-01/WATER
Phase envelope 1 (PRES = 1.01325 bar)
Tie line 1 (PRES = 1.01325 bar)
Tie line 2 (PRES = 1.01325 bar)
Tie line 3 (PRES = 1.01325 bar)
Tie line 4 (PRES = 1.01325 bar)
Tie line 5 (PRES = 1.01325 bar)
Azeotrope 6 (PRES = 1.01325 bar)
Mole fraction of water
Mole fraction of palmitoleyl alcohol
Mole fraction of dodecane

(d)

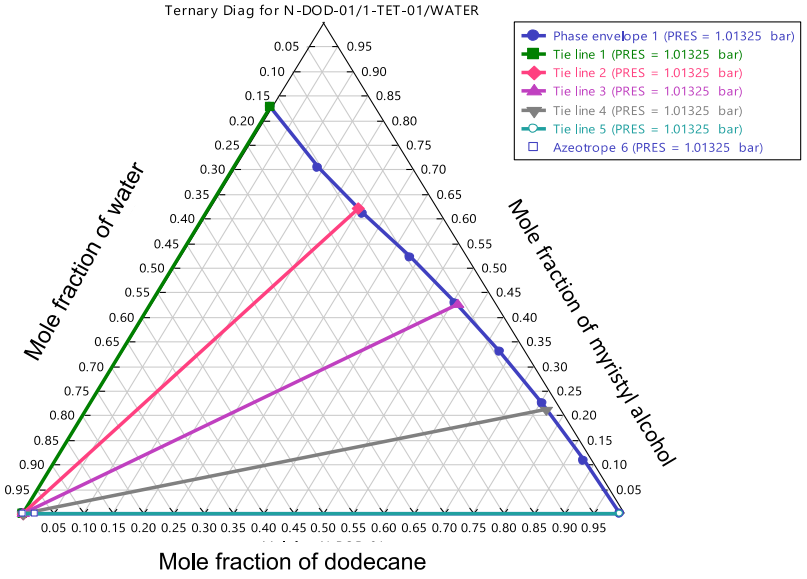


Figure S3. The ternary phase diagrams for (a) Water-oleyl alcohol-dodecane, (b) Water -cetyl alcohol-dodecane, (c) Water-palmitoleyl alcohol-dodecane, and (d) Water-myristyl alcohol-dodecane.

S6. Results of glucose depletion over time

The curves of the glucose concentrations over time are shown in Figure S4. The red and blue lines represent the results from the bioconversion with dodecane layering (dodecane-to-buffer ratio of 1:10) and without dodecane layering, respectively. The results demonstrated that the extractive bioconversion could enable more rapid conversion. However, both experiments showed that the glucose was completely depleted at 12 h.

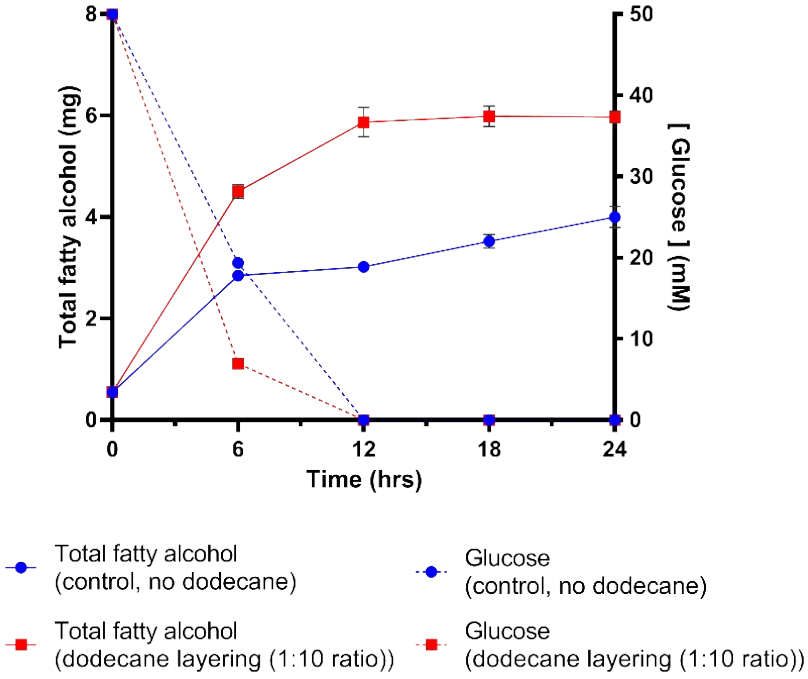


Figure S4. Fatty alcohol production and glucose depletion. The line represents the total amount of fatty alcohol produced (left y-axis). The dashed line represents the glucose concentrations remaining in the buffer phase (right y-axis). The data represent the mean ± SD (n=3).

S7. Results of cell viability

The number of viable cells were estimated using a drop plate technique to determine colony forming units (CFUs). The cell viability (unit: CFU/mL) of different experiments are shown in Figure S5. All experiments, except the one with buffer change, showed decreasing cell viability over time. With the buffer change, the acetate level was thus kept low, increasing the chances for cell survival.

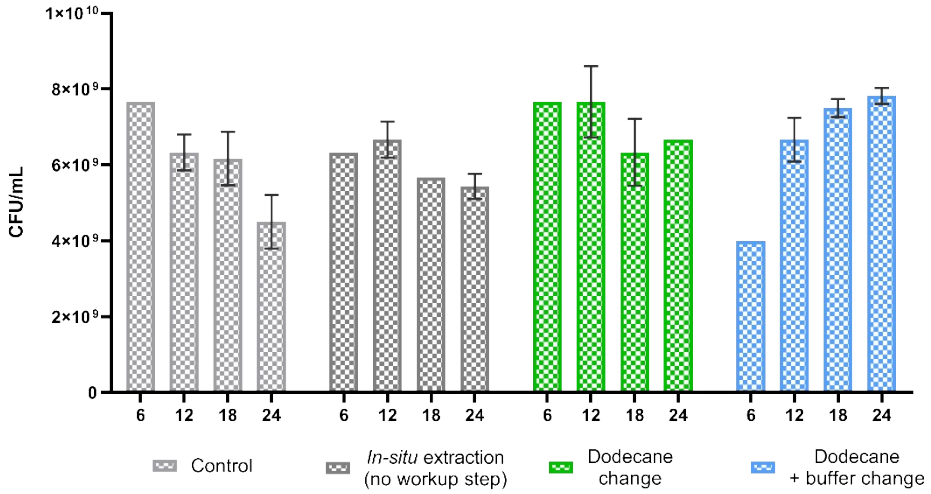


Figure S5. Cell viability over time. The bar graph represents the cell viability (CFU/mL). Control is the condition without dodecane layering. Data represent the mean ± SD (n=3).

S8. Results of product compositions from different experiments

Four types of fatty alcohols were produced during our extractive bioconversion. They were oleyl alcohol (18:1), cetyl alcohol (16:0), palmitoleyl alcohol (16:1), and myristyl alcohol (14:0). Figure S6 shows the % fatty alcohol compositions in different cycles of different experiments. Figure S7 shows the % fatty alcohol composition when they were produced at different temperatures. Palmitoleyl alcohol (16:1) is a major product when the bioconversion is 25°C, which was the temperature used throughout this work.

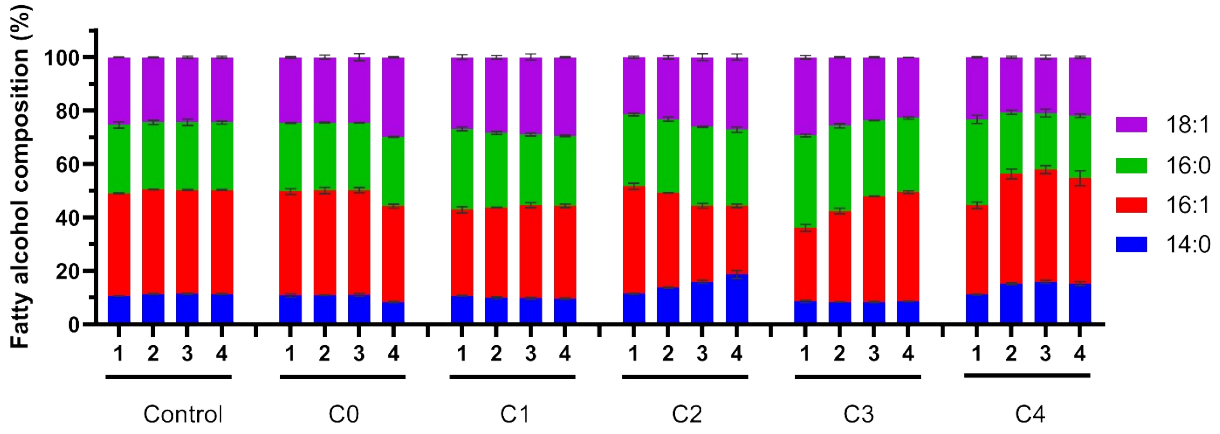


Figure S6. The composition of fatty alcohols produced at different cycles and configurations (control, C0, C1, C2, C3, and C4). Data represent the mean ± SD (N=3).

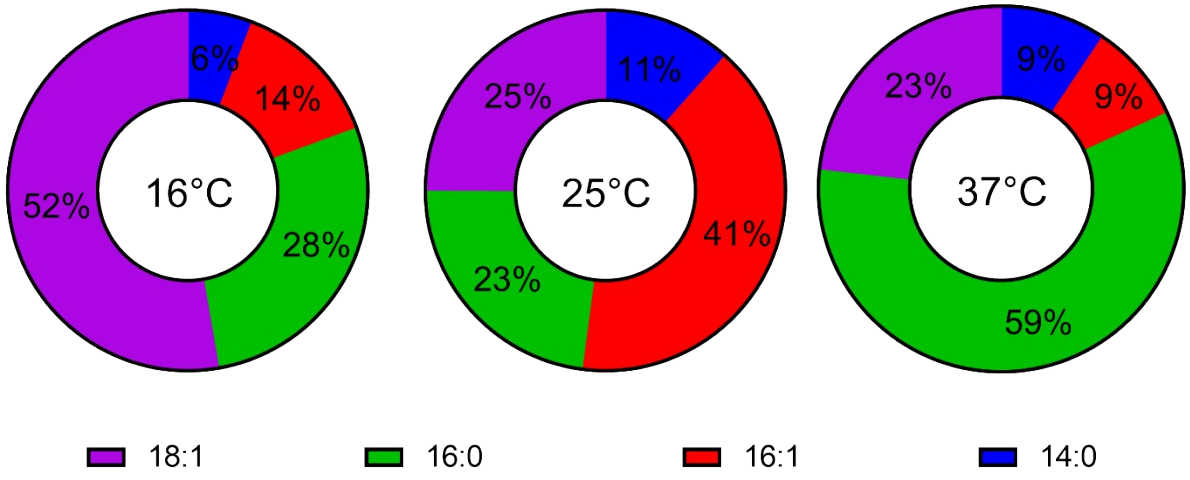


Figure S7. The percent composition of fatty alcohols produced at different temperatures of the bioconversion (16°C, 25°C and 37°C).

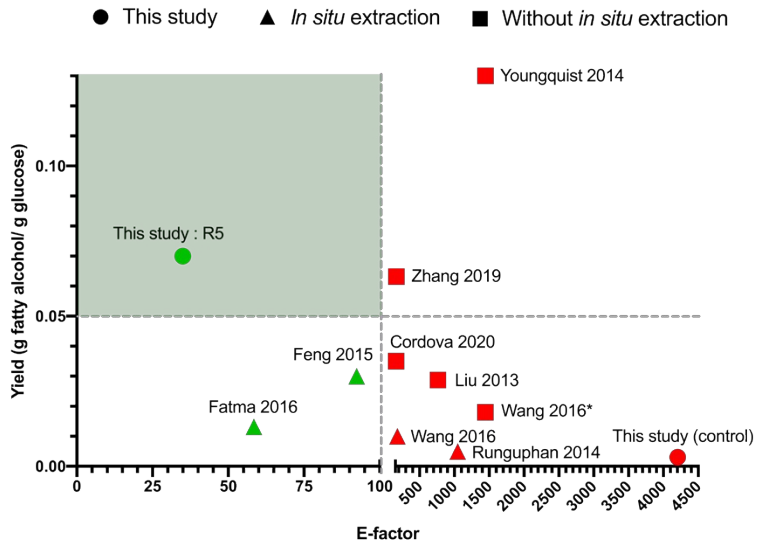
S9. Results for E-factor and complete E-factor values of this work and previous literatures

The E-factors and cE-factors of the previous literatures (Table S4) were estimated using the information in the method section of each work. For the works without *in situ* extraction, the solvent (e.g., ethyl acetate) used to extract dodecane after the bioconversion were taken into account. The yields and E-factors and cE-factors are shown in Figure S8. Unless reported, the yields from the previous literatures were estimated by assuming complete consumption of glucose and other relevant carbon sources.

Table S4. E-factor contribution values of waste for the process of fatty alcohol microbial production under different conditions and relative contribution (%).

References	*In situ* extraction	E-factor or cE-factor contribution of each component (% relative contribution)			E-factor	cE-factor
		Layering or extraction solvent	Buffer	Water		
Liu et al 2013 [1]	Without	729.62 (96%)	31.68 (4%)	-	761.30	-
		729.62 (54%)	31.68 (3%)	579.71 (43%)	-	1,341.01
Fatma et al 2016 [2]	With	37.68 (67%)	18.15 (33%)	-	55.84	-
		37.68 (7%)	18.15 (3%)	502.77 (90%)	-	558.60
Runguphan et al 2014 [3]	With	764.79 (73%)	280.51 (27%)	-	1,045.31	-
		764.79 (7%)	280.51 (2%)	10,204.08 (91%)	-	11,249.39
Feng et al 2015 [4]	With	68.14 (74%)	24.27 (26%)	-	92.41	-
		68.14 (7%)	24.27 (2%)	909.09 (91%)	-	1,001.50
Wang et al 2016 [5]	With	116.80 (64%)	64.94 (36%)	-	181.74	-
		116.80 (8%)	64.94 (4%)	1,298.70 (88%)	-	1,480.44
Wang et al 2016* [6]	Without	1,366.67 (95%)	75.76 (5%)	-	1,442.42	-
		1,366.67 (46%)	75.76 (3%)	1,515.15 (51%)	-	2,957.58
Zhang et al 2019 [7]	Without	156.87 (95%)	8.70 (5%)	-	165.57	-
		156.87 (46%)	8.70 (3%)	173.91 (51%)	-	339.48
Youngquist et al 2014 [8]	With	1,436.74 (99%)	8.03 (1%)	-	1,444.78	-
		1,436.74 (69%)	8.03 (1%)	625 (30%)	-	2,069.78
Cordova et al 2020 [9]	Without	155.52 (97%)	4.74 (3%)	-	160.26	-
		155.52 (47%)	4.74 (1%)	172.41 (52%)	-	332.67
This work (C2)	With	402.74 (98%)	9.94 (2%)	-	412.68	-
		402.74 (37%)	9.94 (1%)	671.68 (62%)	-	1,084.36
This work (C3)	With	383.65 (84%)	75.76 (16%)	-	459.40	-
		383.65 (7%)	75.76 (1%)	5,118.69 (92%)	-	5,578.09
This work (R1)	With	237.27 (95%)	11.71 (5%)	-	248.98	-
		237.27 (23%)	11.71 (1%)	791.43 (76%)	-	1,040.42
This work (R2)	With	194.90 (94%)	12.83 (6%)	-	207.73	-
		194.90 (18%)	12.83 (1%)	866.79 (81%)	-	1,074.52
This work (R3)	With	142.87 (91%)	14.11 (9%)	-	156.98	-
		142.87 (13%)	14.11 (1%)	953.12 (86%)	-	1,110.09

This work (R4: 0.8 mmol)	With	98.68 (84%)	19.49 (16%)	-	118.17	
		98.68 (7%)	19.49 (1%)	1,316.66 (92%)		1,434.83
This work (R4: 1.6 mmol)	With	75.86 (84%)	14.98 (16%)	-	90.84	
		75.86 (7%)	14.98 (1%)	1,012.15 (92%)		1,102.99
This work (R4: 3.0 mmol)	With	119.54 (84%)	23.60 (16%)	-	143.14	
		119.54 (7%)	23.60 (1%)	1,594.90 (92%)		1,738.04
This work (R4: 4.0 mmol)	With	115.66 (84%)	22.84 (16%)	-	138.50	
		115.66 (7%)	22.84 (1%)	1,543.21 (92%)		1,681.71
This work (R5: 4.0 mmol)	With	29.21 (84%)	5.77 (16%)	-	34.98	
		29.21 (7%)	5.77 (1%)	389.71 (92%)		424.69
This work (Control: 0.8 mmol)	Without	1,973.74 (98%)	32.39 (2%)	-	2,006.12	-
		1,973.74 (47%)	32.39 (1%)	2,188.18 (52%)	-	4,194.31
This work (Control: 4.0 mmol)	Without	4,137.99 (98%)	67.90 (2%)	-	4,205.89	
		4,137.99 (47%)	67.90 (1%)	4,587.58 (52%)		8,793.47



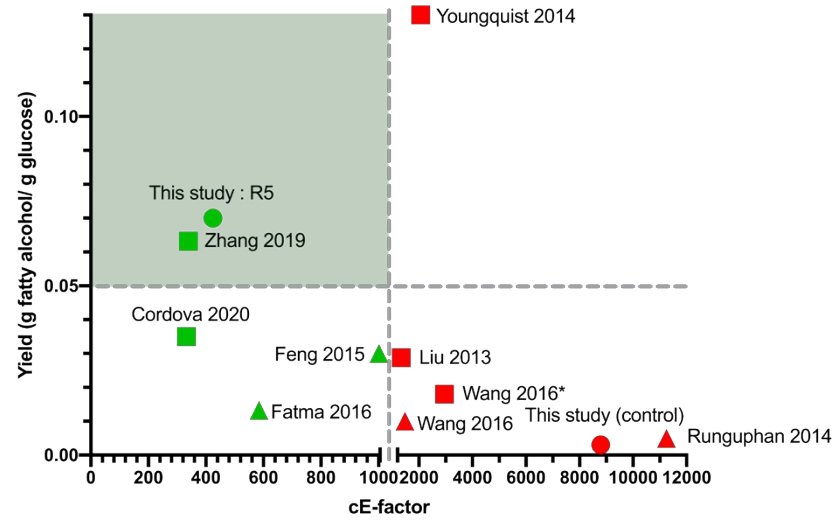


Figure S8. Production yields with E-factors (Top) and cE-factors (Bottom) of the processes developed in this work and previous reports.

S10. Results of the productions in 8-cycle bioconversion

Figure S9 shows the fatty alcohol productions in the conventional semi-batch (C2) and multiple sequential batch (C4) operations comparing to the productions in the reconfigurations R4 and R5 as presented in this work. Note that the amount of the total glucose added was 0.8 mmol for C2, C4, and R4, and 4.0 mmol for R5. R5 allows for the production with a high glucose concentration as the glucose addition was distributed throughout the course of the eight cycles.

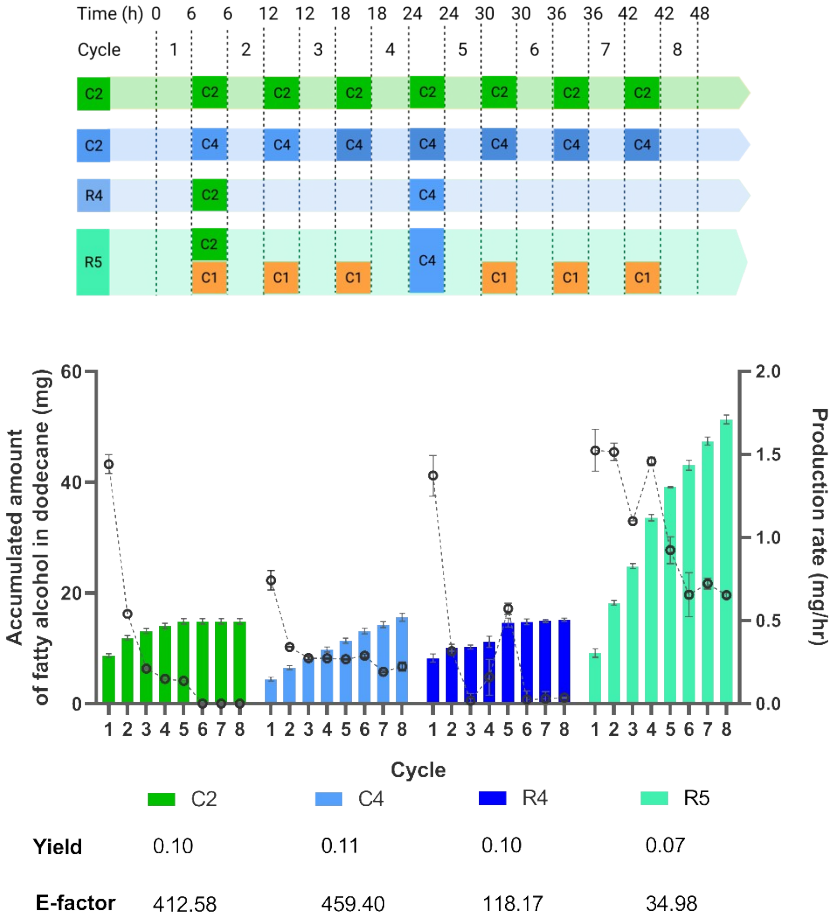


Figure S9. Fatty alcohols production in different experiments (C2, C4, R4, R5) with the accumulated amount of fatty alcohol in the dodecane phase (column) and production rate (line) in each cycle. The experimental conditions: initial OD_{600} = 30, with the total glucose addition = 0.8 mmol for C2, C4, R4 and 4 mmol for R5. Bioconversion was performed at 25°C with shaking at 250 rpm and for 48 h in total (6 h/cycle). Each data point was collected from three separate, repeated experiments (triplicates).

S11. Results of pH from different configurations

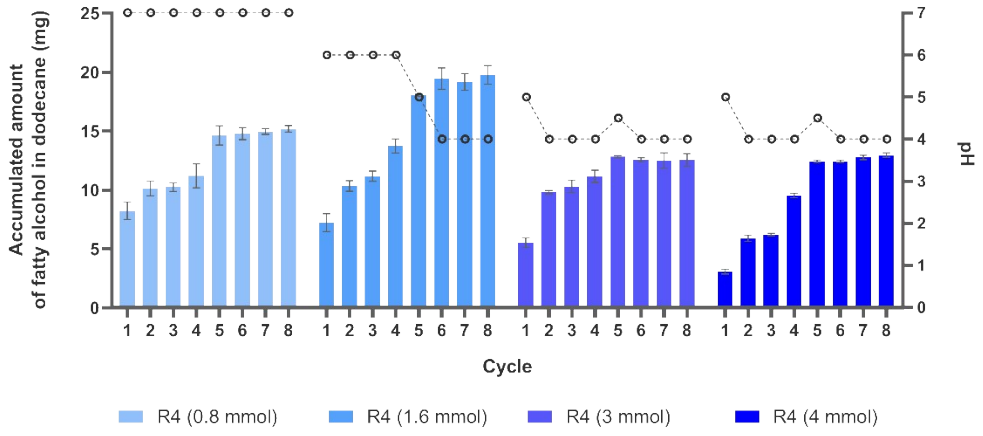


Figure S10. Fatty alcohols production using the reconfigurations R4 at different total glucose additions (0.8, 1.6, 3, and 4 mmol). The graph presents the accumulated amount of fatty alcohol in the dodecane phase (column) and pH (line) in each cycle. The experimental conditions: initial OD_{600} = 30. Bioconversion was performed at 25°C with shaking at 250 rpm and for 48 h in total (6 h/cycle). Each data point was collected from three separate, repeated experiments (triplicates).

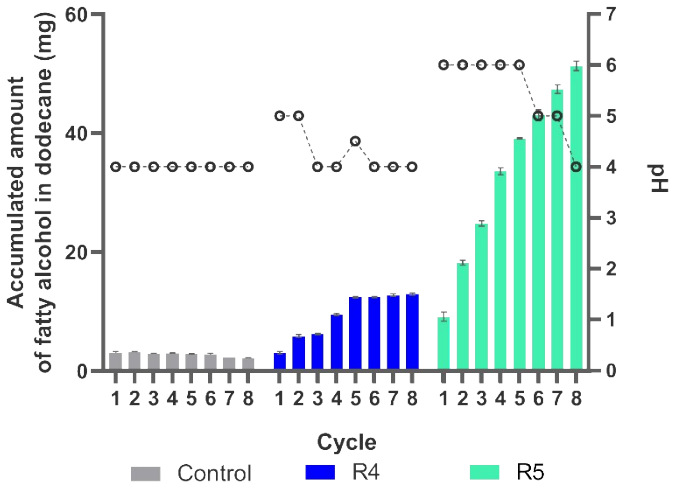


Figure S11. Fatty alcohols production using the control (without *in situ* extraction) and the reconfigurations R4 and R5. The graph presents the accumulated amount of fatty alcohol in the dodecane phase (column) and pH (line) in each cycle. The experimental conditions: initial OD_{600} = 30 and 4.0 mmol glucose (total). Bioconversion was performed at 25°C with shaking at 250 rpm and for 48 h in total (6 h/cycle). Each data point was collected from three separate, repeated experiments (triplicates).

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