Electronic supplementary information (ESI) for the article:

Integrating the opposites of biofuel production: absorption of short-chain alcohols into oleaginous yeast cells for butanol recovery and wet-extraction of microbial oil

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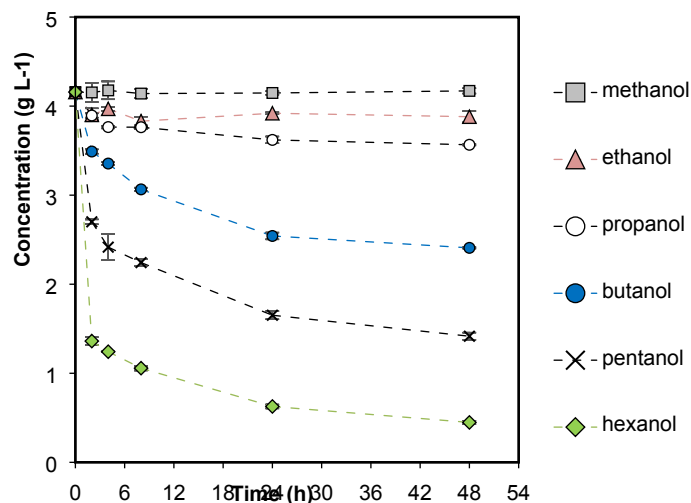
Figure S1. Time-dependent changes in water concentration of alcohols in suspensions containing 37.8 g L-1 of oleaginous yeast *R. fluviale*. Initial alcohol concentration in the aqueous phase was 4.16 g L-1 and the suspensions were agitated at 50 °C in gas-tight headspace vials. The error bars indicate average deviations from the mean value (N=2).
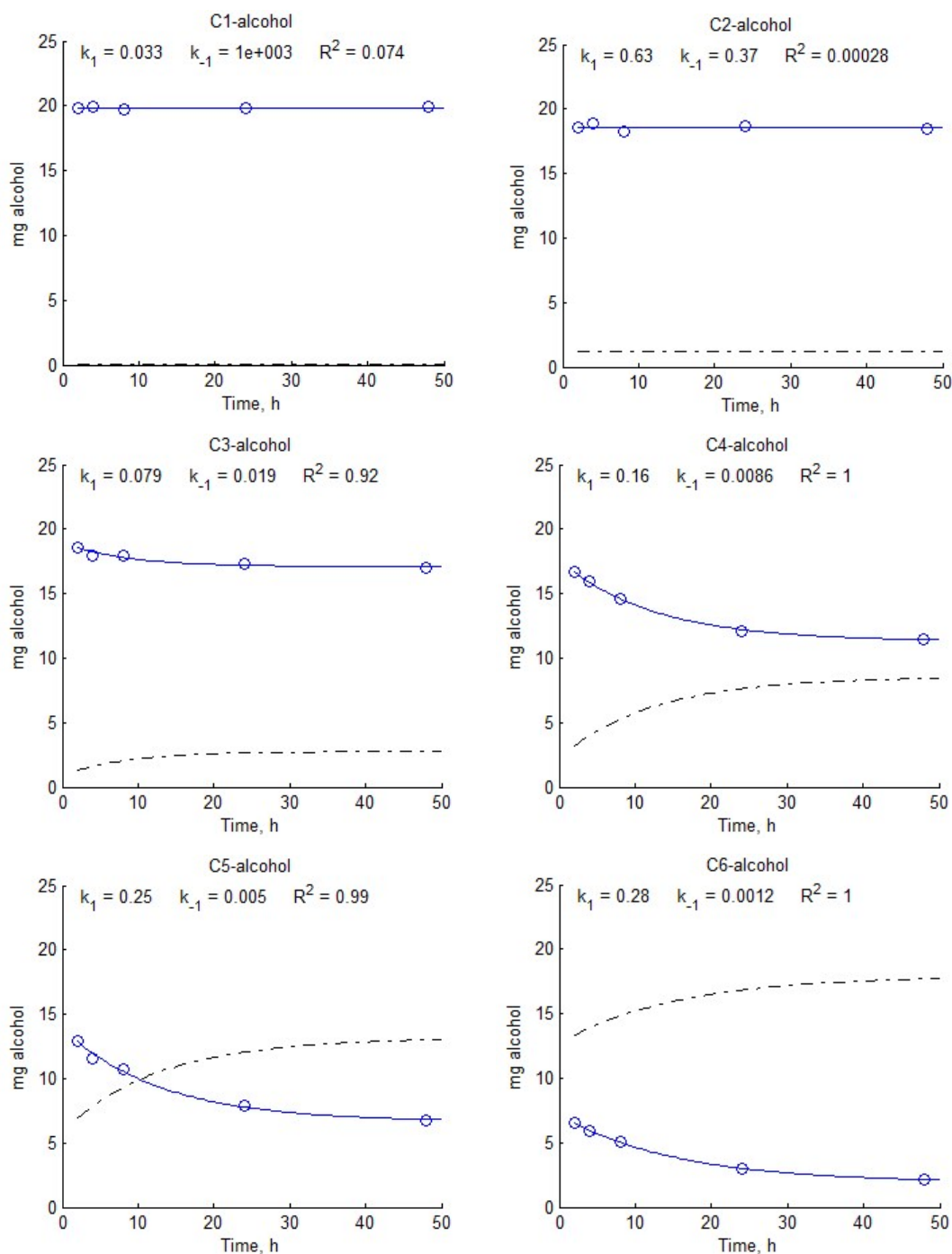
Figure S2. First order kinetic model fitted to the slow stage of absorption of 1-alcohols from water into oleaginous yeast *R. fluviale* containing 69% total fatty acid content relative to cell dry weight (CDW). Partition of the alcohols (20 mg initial amount) into the CDW is shown by the dashed line, and the remaining alcohol in the water phase is indicated by the blue markers and the continuous lines. The rapid absorption took place instantaneously, with approximately half of the total amount of 1-hexanol absorbed during the first 5 minutes.
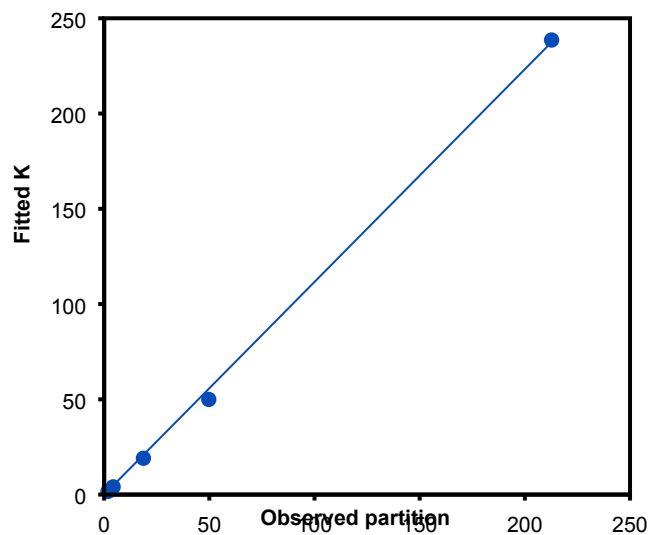
Figure S3. Comparison of the fitted equilibrium constants to the observed partition of 1-alcohols from water to oleaginous yeast *R. fluviale* containing 69% total fatty acid content relative to cell dry weight. Partition of the 1-alcohols from methanol to hexanol were determined. The line shows a linear fit to the data.
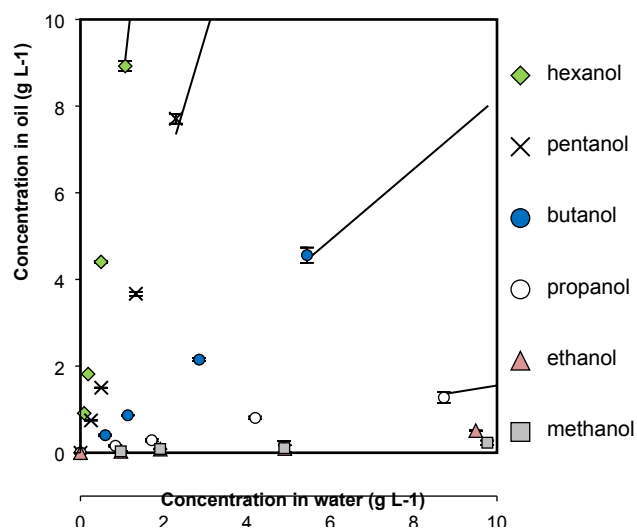
Figure S4. Partition of alcohols at 50 °C in biphasic rapeseed oil/water mixtures. The mixtures were subjected to vigorous agitation during 20 h, and the concentrations were determined after complete phase settling. The error bars are shown for the concentrations in oil, and indicate average deviations from the mean value (N=2).

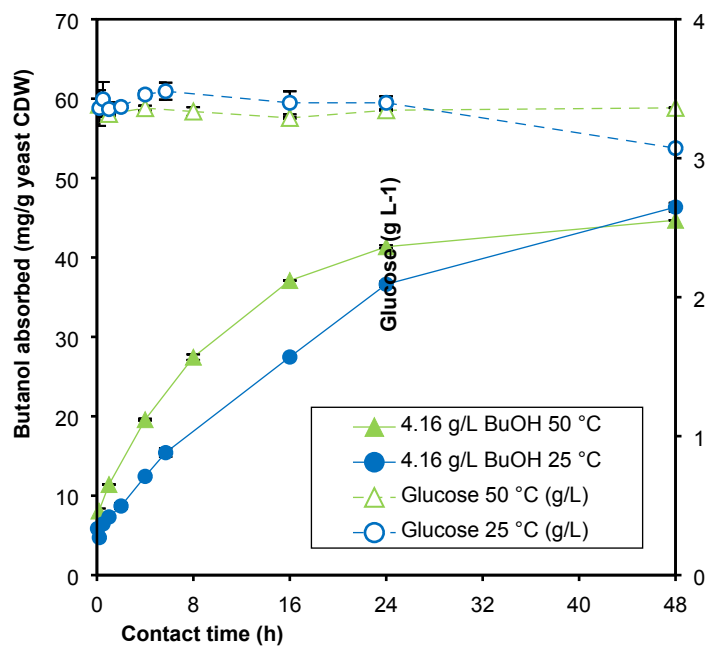**Figure S5.** Absorption of butanol into oleaginous yeast *R. fluviale* cells containing 69% total fatty acid content relative to cell dry weight (CDW). The extent of absorption from water (mg butanol/g yeast CDW) is shown at the temperature of 25 °C and 50 °C, with initial butanol concentration of 4.16 g/L in each. Glucose concentrations were determined simultaneously with butanol using HPLC.
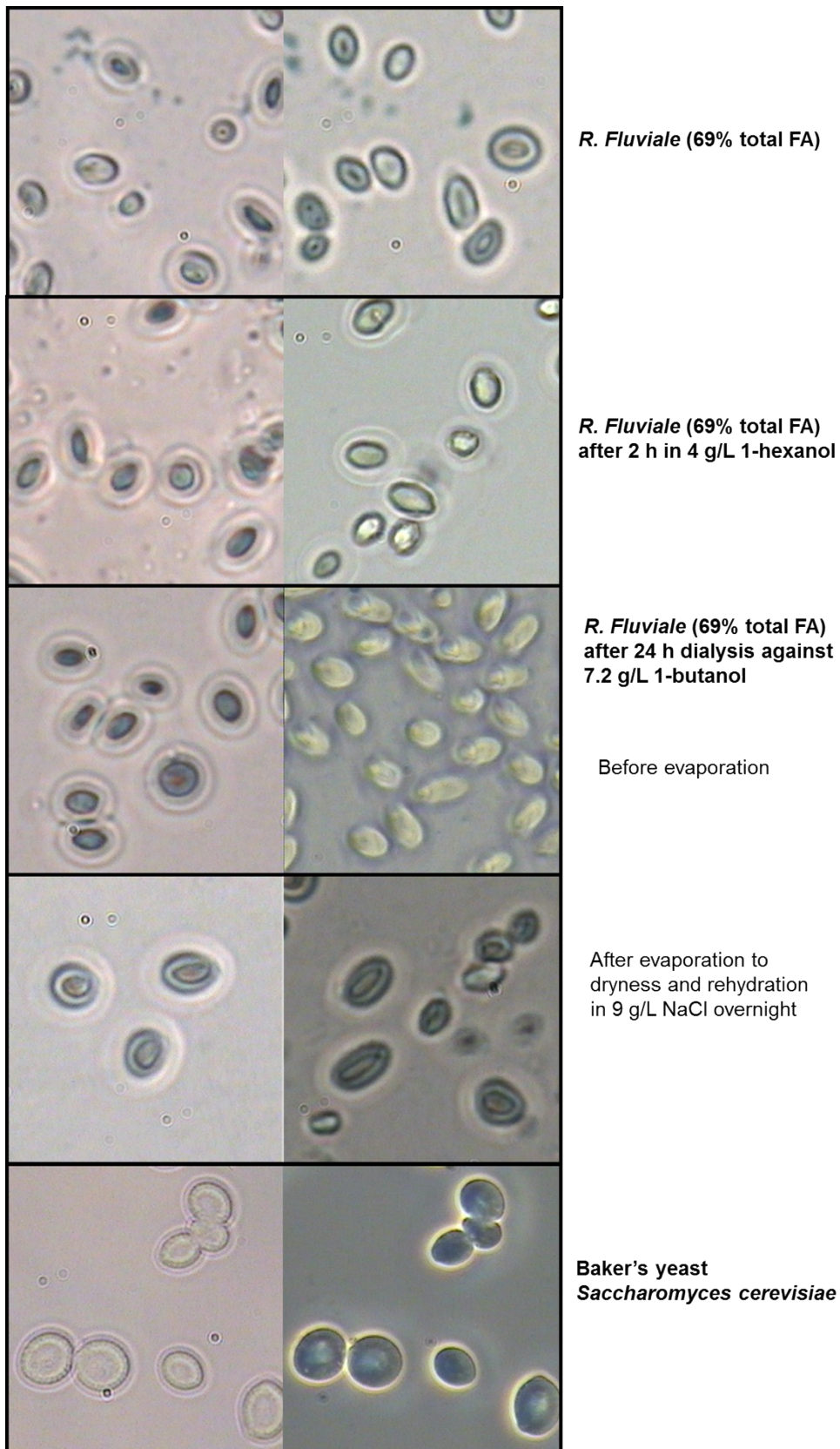
Figure S6. Optical phase contrast micrographs at 1000 × magnification of oleaginous yeast *R. fluviale* and baker's yeast *S. cerevisiae.* Sudan black B staining directly on the microscopy plate.
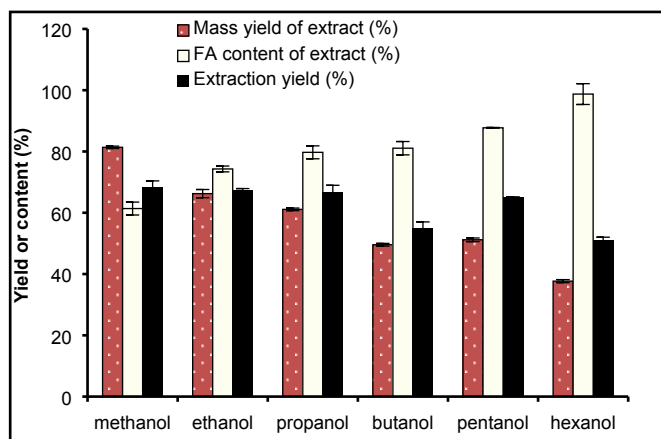
Figure S7. Wet-extraction of oleaginous yeast R. fluviale suspension (1 mL, 189 mg CDW at 69% total FA content) with 1-alcohol (5 mL). Extraction was conducted within 2 h at 80 °C. Mass yield of extract (g dry extract/g yeast CDW), FA content of extract (g FA/g extract), and extraction yield (mass yield of extract·FA content of extract/total FA content of yeast) are shown. The errorbars indicate average deviations from the mean value (N=2).

Absorption of butanol into oleaginous yeast biomass across a semipermeable membrane

An absorption experiment at larger scale was carried out in order to verify the recovery of butanol from the oleaginous yeast cells. For this, 20 mL of *R. fluviale* water suspension that contained 3.78 g of CDW at 69% total FA content was loaded into a dialysis bag (Medicell International Ltd.) having 12−14 kDa molecular weight cut-off. The yeast suspension in the dialysis bag was placed into a conical flask preloaded with 80 mL of water solution of butanol at a concentration of 7.2 g/L. The flask was capped airtight and agitated at 200 rpm during 24 h at 25 °C. The concentration of butanol was determined from the water phase in the interior and the exterior of the dialysis bag. The contents of the dialysis bag were distilled to dryness, and the distillate was diluted in known amount of water for the determination butanol mass balance (Table S1).

Table S1. Partition of 1-butanol in absorption experiment in dialysis bag.

Concentration of 1-butanol (g L^{-1})	0 h	24 h
In the water phase in the exterior of the dialysis bag (direct GC analysis)	7.2	4.4
Calculated in the water phase present in the entire system (homogeneous distribution assumed)	5.9	5.9
In the water phase in the interior of the dialysis bag (direct GC analysis)	0	5.1
In the water phase in the interior of the dialysis bag (calculated based on butanol mass balance, *i.e.* butanol found in the distillate is assumed to be present only in the water)	0	9.8
Mass balance of 1-butanol (g)	**0 h**	**24 h**
In the water phase in the exterior of the dialysis bag (direct GC analysis)	0.57	0.33
In the distillate	0	0.22
Total	0.57	**0.55**

Table S2. Effect of extraction temperature and solvent on (A) gravimetric mass yield of extract, (B) total fatty acid (FA) content of extract and (C) the extraction yield (C=AB/69%).

T (°C)	Extraction solvent	(A) Mass yield of extract (%)	(B) Total FA content of extract (%)	(C) Extraction yield (%)	Fatty acid distribution (% of total FA)					
					C14:0	C16:0	C16:1	C18:0	C18:1	C18:2
25	methanol	80.2 ± 0.7	46.8 ± 0.2	51.4 ± 0.2	1.6	25.0	1.8	3.5	44.0	24.1
	ethanol	68.9 ± 0.7	66.5 ± 3.9	62.7 ± 3.0	1.5	25.0	1.6	3.9	44.7	23.2
	1-propanol	66.1 ± 0.5	72.0 ± 0.1	65.2 ± 0.4	1.4	24.3	1.7	3.8	45.1	23.6
	1-butanol	46.3 ± 0.7	86.0 ± 3.8	54.4 ± 1.6	1.4	25.1	1.6	3.9	44.9	23.0
	1-pentanol	34.2 ± 7.5	113.1 ± 1.2	56.1 ± 0.6	1.4	25.1	1.6	4.0	45.1	22.8
	1-hexanol	60.1 ± 2.0	71.4 ± 2.7	58.7 ± 0.2	1.5	24.6	1.7	3.9	44.9	23.4
80	methanol	81.4 ± 0.4	61.4 ± 2.1	68.4 ± 2.0	1.4	25.0	1.5	4.4	45.5	22.2
	ethanol	66.2 ± 1.3	74.3 ± 1.0	67.4 ± 0.5	1.5	24.7	1.7	3.8	44.9	23.4
	1-propanol	61.1 ± 0.4	79.7 ± 2.1	66.7 ± 2.2	1.6	24.9	1.7	3.6	44.4	23.7
	1-butanol	49.6 ± 0.4	81.1 ± 2.2	55.0 ± 2.0	1.5	24.9	1.7	3.8	44.7	23.4
	1-pentanol	51.2 ± 0.6	87.7 ± 0.1	65.1 ± 0.1	1.5	24.6	1.7	3.6	44.3	24.2
	1-hexanol	37.7 ± 0.5	98.7 ± 3.4	50.9 ± 1.1	1.5	24.2	1.7	3.7	44.7	24.2