**Supplementary materials**

Integrated process for bioenergy production and water recycling in the dairy industry: selection of *Kluyveromyces* strains for direct conversion of concentrated lactose-rich streams into bioethanol

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Table S1. Current recommendations and regulations for drinking water.

| Parameter                        | WHO 1 | EC 2  | EPA 3  | JWWA 4 |
|----------------------------------|-------|-------|--------|--------|
| pH                              | 6.5-8.5* | 6.5-9.5 | 6.5-8.5* | 5.8-8.6 |
| Total dissolved solids (g/L) (180°C) | 0.6*  | 0.5*  | 0.5    |
| Conductivity (µS/cm) (20°C)      | 2500  |       |        |
| Anions (mg/L)                    |       |       |        |
| F⁻                             | 0.5-1* | 1.5   | 4.0/2.0* | 0.8    |
| Cl⁻                             | 250*  | 250   | 250*   | 200    |
| HCO₃⁻                           | 500*  | 250   | 250*   |
| SO₄²⁻                           | 50    | 50    | 10     |
| NO₃⁻                           | 3     | 0.5   | 1      |
| Cations (mg/L)                  |       |       |        |
| Na⁺                             | 200*  | 200   | 200    |
| K⁺                              |       |       |        |
| Mg²⁺                            |       |       |        |
| Ca²⁺                            |       |       |        |
| Fe³⁺                            | 0.3*  | 0.2   | 0.3*   |
| NH₄⁺                            | 1.5*  | 0.5   | 0.3    |
| Mn²⁺                            | 0.1*  | 0.05  | 0.05*  |
| Vestigial Elements (µg/L)        |       |       |        |
| Cu                              | 2000  | 2000  | 1300   | 1000   |
| Zn                              | 4000* |       | 5000*  | 1000   |
| Cd                               | 3     | 5     | 5      |
| Pb                               | 10    | 10    | 15     |
| Hg                               | 6     | 1     | 2      |

* recommended values according to aesthetic acceptability thresholds; 1 World Health Organization (WHO), (2017). Guidelines for Drinking-water Quality. Technical Report (https://apps.who.int/iris/bitstream/handle/10665/254637/9789241549950-eng.pdf); 2 European Commission (EC), (1998). COUNCIL DIRECTIVE 98/83/EC of 3 November 1998 on the quality of water intended for human consumption. Technical Report (https://eur-lex.europa.eu/legal-content/EN/TXT/PDF/?uri=CELEX:31998L0083&from=EN); 3 United States Environmental Protection Agency (EPA), (2017). Ground Water and Drinking Water - National Primary Drinking Water Regulations. Technical Report (https://www.epa.gov/ground-water-and-drinking-water/national-primary-drinking-water-regulations#Inorganic; https://www.epa.gov/dwstandardsregulations/secondary-drinking-water-standards-guidance-nuisance-chemicals); 4 Japan Water Works Association (JWWA), (2007). Supply of Drinking Water with Clean and Safe - Water Quality Standards of Drinking Water. Technical Report (http://www.jwwa.or.jp/english/water_en/water-e07.html).
### Table S2. Properties and composition of reference defined media for yeast cultivation.

| Main components       | YNB ¹   | Verduyn ²  | Delft ³  |
|-----------------------|---------|-----------|---------|
| D-Glucose (g/L)       | 5       | 10        | 22      |
| pH (25°C)             | 5.4     | 5.0       | 6.0     |
| **Anions (mg/L)**     |         |           |         |
| F⁻                    | -       | -         | -       |
| Cl⁻                   | 125     | 0.4       | 0.9     |
| HCO₃⁻                 | -       | -         | -       |
| SO₄²⁻                 | 3827    | 3829      | 5657    |
| H₂PO₄⁻                | 708     | 2134      | 10262   |
| NO₃⁻                  | -       | -         | -       |
| NO₂⁻                  | -       | -         | -       |
| MoO₄²⁻                | 0.2     | 0.3       | 0.6     |
| BO₃⁻                  | 0.5     | 0.9       | 1.9     |
| **Cations (mg/L)**    |         |           |         |
| Li⁺                   | -       | -         | -       |
| Na⁺                   | 39      | 0.1       | 0.2     |
| K⁺                    | 285     | 860       | 4137    |
| Mg²⁺                  | 493     | 493       | 493     |
| Ca²⁺                  | 36      | 1.2       | 2.5     |
| Fe (total)            | 0.1     | 0.6       | 1.2     |
| NH₄⁺                  | 1361    | 1361      | 2045    |
| Mn²⁺                  | 0.2     | 0.3       | 0.5     |
| **Vestigial Elements (μg/L)** |           |           |         |
| Cu                    | 16      | 64        | 127     |
| Zn                    | 131     | 1020      | 2041    |
| Co                    | -       | 59        | 118     |

¹ Difco™ Yeast Nitrogen Base (without amino acids) (http://legacy.bd.com/europe/regulatory/Assets/IFU/Difco_BBL/233520.pdf); ² Verduyn, C., Postma, E., Scheffers, W. A., & Van Dijken, J. P. (1992). Effect of benzoic acid on metabolic fluxes in yeasts: A continuous-culture study on the regulation of respiration and alcoholic fermentation. Yeast, 8(7), 501-517. doi: 10.1002/yea.320080703; ³ Jensen, N. B., Strucko, T., Kildegaard, K. R., David, F., Maury, J., Mortensen, U. H., Borodina, I. (2014). EasyClone: method for iterative chromosomal integration of multiple genes in *Saccharomyces cerevisiae*. FEMS Yeast Res, 14(2), 238-248. doi: 10.1111/1567-1364.12118
Figure S1. Effect of ethanol and lactose concentration on the lactose consumption rate by *K. marxianus* PYCC 3282. Lactose consumption by strain *K. marxianus* PYCC 3282 (CBS 608) in rich media YP with 20 g/L lactose (Lac20) and YP with 40 g/L lactose (Lac40) media, with and without addition of ethanol: initial ethanol concentrations 10 g/L (E10) or 20 g/L (E20). Error bars represent standard deviation from the average value of two independent experiments. **a**, Lactose consumption. **b**, apparent lactose consumption rates (Time points used for calculations: Lac20 media, from 2 h to 5 h; Lac40 media, from 5 h to 24 h). Cells were grown for 24 h in YPD medium, harvested by centrifugation (10,414 g at 4°C for 10 min), washed twice with cold sterile water and used to inoculate 10 mL of sterile medium, at an initial cell density of 2.7 ± 0.1 gCDW/L. Cells were cultivated in shake flasks (volume ratio medium/flask 1:5), with cotton plugs, in an orbital shaker (Agitorb 200, Aralab) at 30°C, with 150 rpm agitation. Assays were performed in duplicate.