**Design of a stable ethanologenic bacterial strain without heterologous plasmids and antibiotic resistance genes for efficient ethanol production from concentrated dairy waste**

***Supplementary figures***

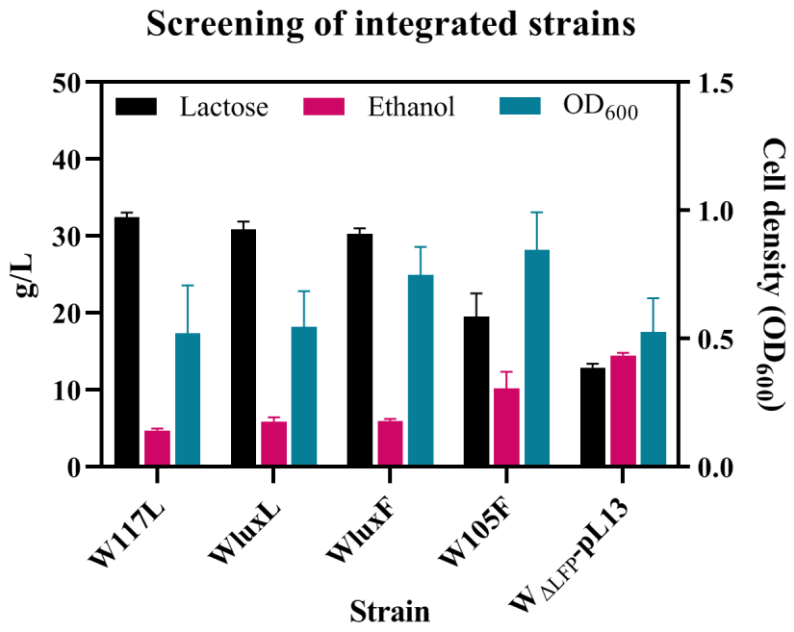


**Figure S1.** Fermentation performance screening in $CWP_{R1:2}$ + PIPES in test tube experiments for the four new ethanologenic strains constructed in this work with a chromosomally integrated *adhB-pdc* operon. Bars indicate the average lactose concentration, ethanol concentration and optical density at 600 nm ($OD_{600}$) after 72 h, for at least three replicates in the same day, with error bars corresponding to standard deviations. $OD_{600}$ was used as a measure of cell density and was obtained by transferring 200 µl of properly diluted cultures into a 96-well plate and absorbance was acquired using an Infinite F200Pro plate reader (Tecan). Raw absorbance values were background-subtracted using $CWP_{R1:2}$ + PIPES and multiplied by their dilution factor to obtain the final $OD_{600}$ value. Data for the plasmid-based $W_{\Delta LFP}$-pL13 strain, tested in parallel with the four candidate strains, are shown as a reference. Initial lactose concentration was 48.3 g/L.

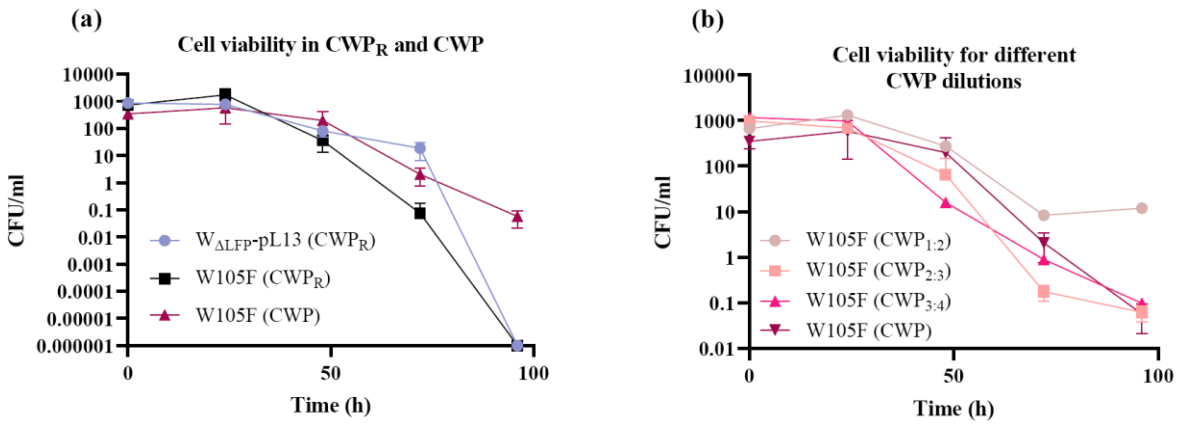


**Figure S2.** Cell viability time course for the W105F strain in different wastewaters, CWP$_R$ or CWP (a) and in CWP at different dilutions (b) in terms of CFU/ml. Data points represent values from a single replicate or the average of two replicates (as reported in Table 2 in the main text), with error bars corresponding to the standard deviation. In panel (a), CFU/ml data are also shown for the plasmid-based W$_{\Delta LFP}$-pL13 strain, used as a reference.

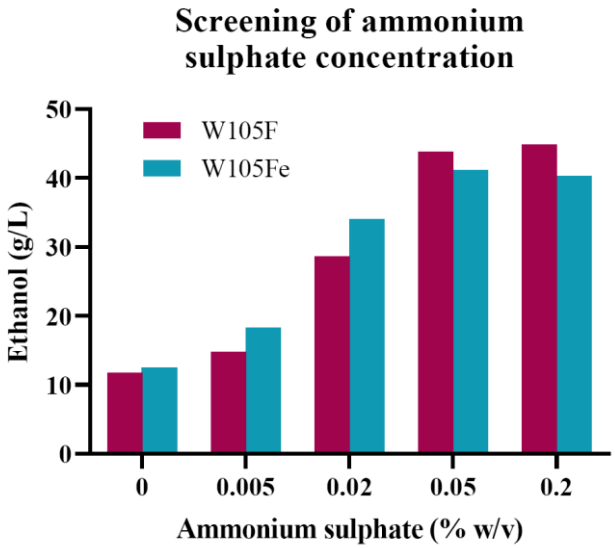


**Figure S3.** Fermentation performance screening in CWP + PIPES in test tube experiments for the W105F and W105Fe strains with different concentrations of ammonium sulphate. Bars indicate the ethanol concentrations of single replicates after 72 h.

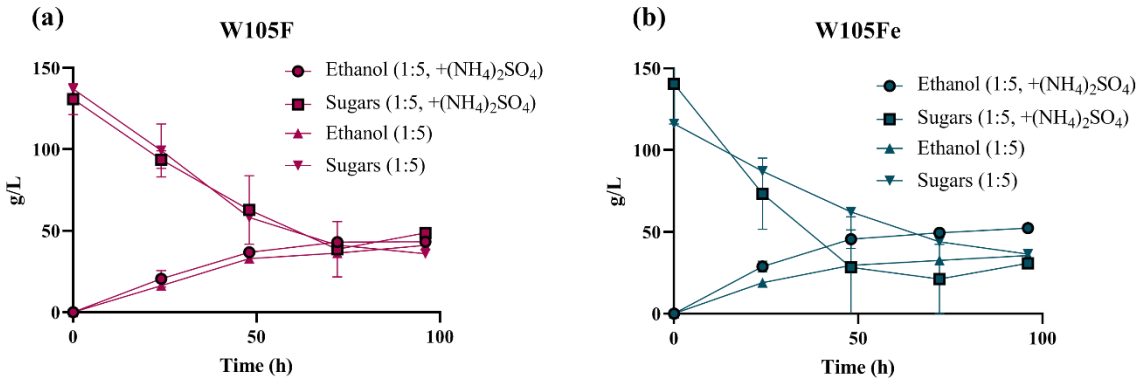


**Figure S4.** Performance comparison of W105F (a) or W105Fe (b) between the ammonium sulphate-supplemented CWP and non-supplemented CWP conditions, all of them with 1:5 inoculum ratio. Fermentation time course data from pH-controlled bioreactor experiments are represented as concentration time series of sugars and ethanol. The reported profiles are also available in Figure 1 and Figure 3 of the main text, and are herein shown in the same plots to support pairwise comparison. Data points represent values from a single replicate or the average of two replicates (as reported in Table 2), with error bars corresponding to the standard deviation.