Supplementary Material to: Engineering the outcome of cofermentation processes by altering the feedstock sugar-to-protein ratio

R. Bevilacqua*, M. Mauricio-Iglesias, S. Balboa, J.M. Lema, M. Carballa

CRETUS, Department of Chemical Engineering, Universidade de Santiago de Compostela, 15782 Santiago de Compostela, Spain – riccardo.bevilacqua@usc.es

MATERIALS AND METHODS

Calculations

The acidification degree was calculated based on the concentration of measured VFA (in this case aliphatic VFA) and expressed as:

$$Acidification\ degree\ (\%) = \frac{\Sigma\ C_{VFA}}{C_{pr}} \times\ 100 \quad (1)$$

where C_{VFA} stands for the total concentration of the measured VFAs (in g COD-VFA/L) in the reactor effluent and C_{pr} for the total protein concentration (in g COD/L) in the feeding of the reactor.

Ammonification was also used as a proxy to monitor protein conversion to VFA, as amino acid fermentation is always related to NH4+ release. It was expressed as follows:

$$Ammonification\ (\%) = \frac{C_{TANeffluent} - C_{TANfeeding} + C_{TANbiomass}}{C_{TANmaximum}} \times\ 100 \quad (2)$$

Where $C_{TANeffluent}$ is the concentration of ammonium nitrogen (mg N-NH_4^+/L) measured in the reactor effluent, $C_{TANfeeding}$ is the concentration of ammonium nitrogen (mg N-NH_4^+/L) in the reactor feeding derived from the macronutrients supplementation, $C_{TANbiomass}$ is the concentration of ammonium nitrogen (mg N-NH_4^+/L) which was captured by biomass during growth and $C_{TANmaximum}$ is the

maximum concentration of ammonium nitrogen (mg N-NH_4^+/L) achieved if complete conversion of the protein to VFA occurs. $C_{TANbiomass}$ was calculated by multiplying the measured in-reactor biomass concentration for a biomass nitrogen ratio of 114 mgN-NH_4^+/g VSS, assuming an average biomass composition of $CH_{1.8}O_{0.5}N_{0.2}$. $C_{TANmaximum}$ was estimated based on Total Kjeldahl Nitrogen measurements (SM4500C) of casein, which yielded a mg N-NH_4^+/g protein ratio of 171.94.

RESULTS AND DISCUSSION

3.4 The influence of STP on the microbial community structure

Table S1. Data summary and Alpha diversity indexes. Total reads indicate the number of reads after low-quality data trimming and chimera removal. OTUs correspond to the number of OTUs above 1% of abundance.

Day	STP ratio	Total reads	OTUs	Diversity indexes	
				Shannon	Simpson
63	0.25	33,874	98	4.19	0.9
70	0.25	34,343	95	3.84	0.88
77	0.25	32,006	94	3.99	0.89
218	0.50	34,544	92	3.68	0.86
225	0.50	36,889	66	2.68	0.69
232	0.50	28,266	94	3.87	0.88
246	0.75	41,517	94	3.45	0.78
253	0.75	42,916	90	3.51	0.8
260	0.75	34,131	92	3.85	0.87
330	1.0	36,292	83	2.11	0.64
337	1.0	39,111	79	1.91	0.54
344	1.0	46,105	89	2.52	0.68
81	2.0	33,455	76	3.05	0.78
84	2.0	42,174	69	2.68	0.74
88	2.0	32,062	67	2.52	0.7

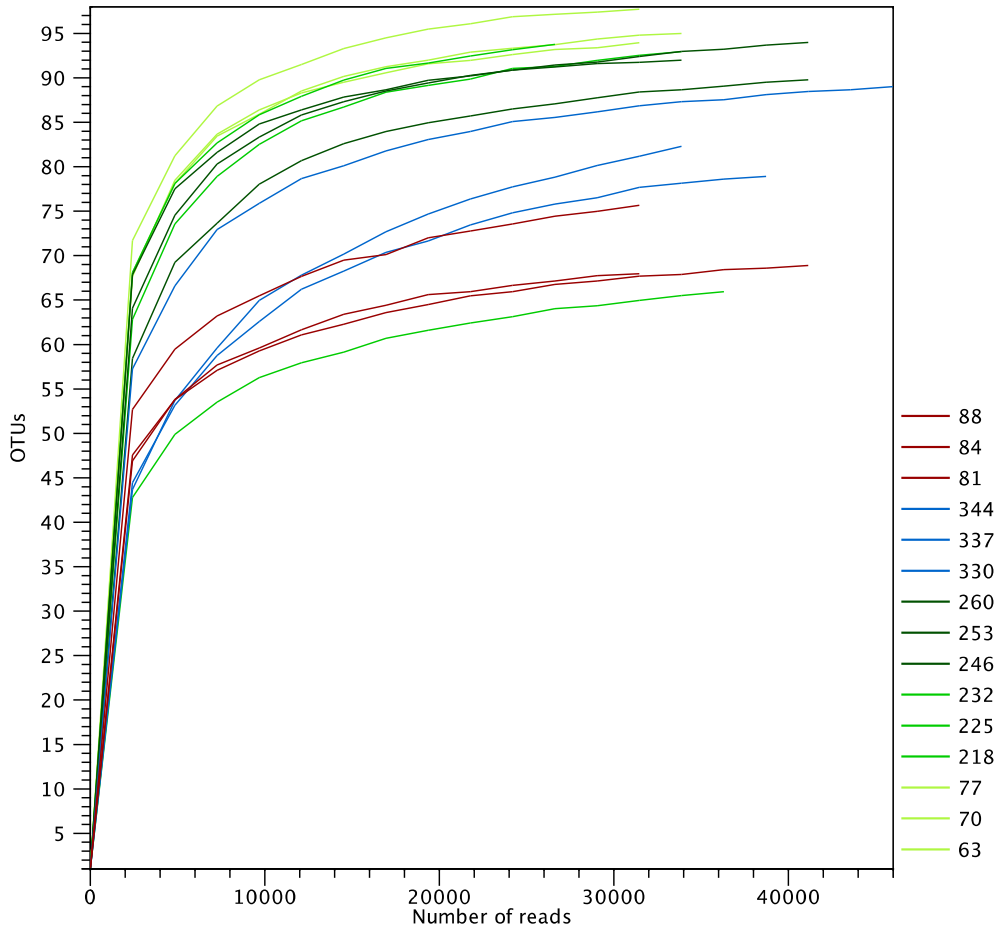

Figure S1. Rarefaction curves of OTUs. The x-axis represents the number of reads per sample and the y-axis the number of observed OTUs. Curves represent each sample and are coloured according to the STP ratio: 0.25 (light green), 0.5 (medium green), 0.75 (dark green), 1.0 (blue) and 2.0 (red).

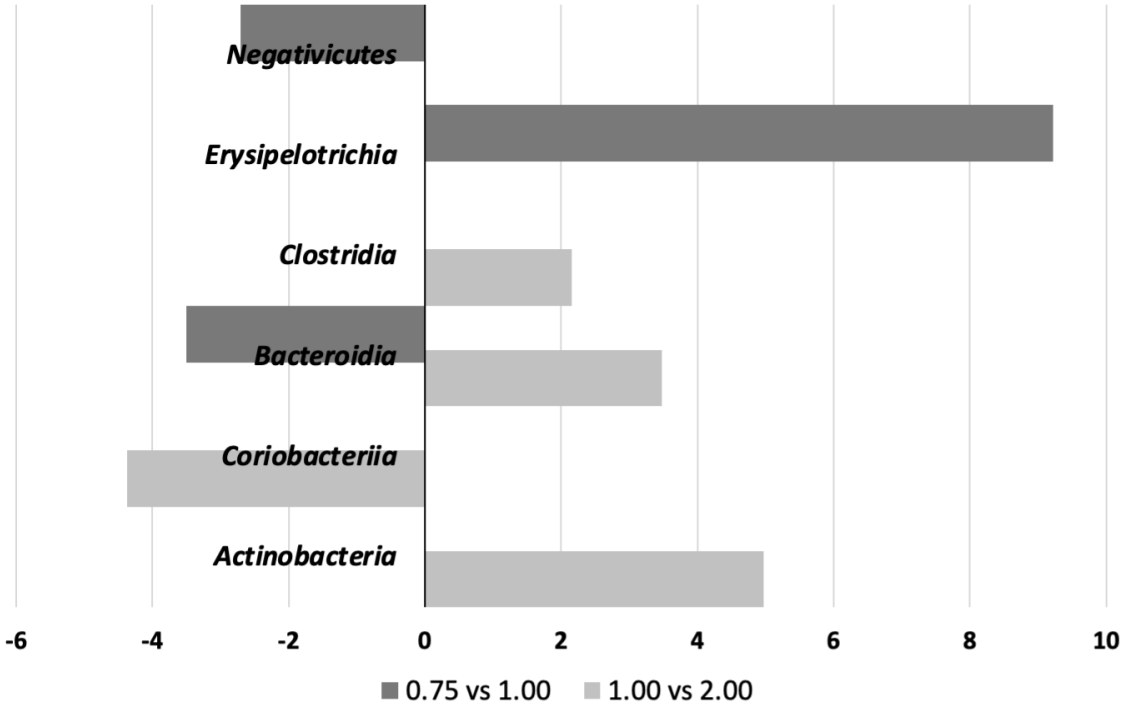

Figure S2. Significant differential abundance (log2 fold change) of the most abundant bacterial classes between STP ratios of 0.75 and 1.0 (dark grey) and between the STP ratios of 1.0 and 2.0 (light grey).

References

1. APHA. Standard Methods for the Examination of Water and Wastewater. *America Public Health Association,* 2017, Washington, DC, USA.
2. S.A. Cohen, K. De Antonis, D.P. Michaud. Compositional protein analysis using 6-aminoquinolyl-N-hydroxysuccinimidyl carbamate, a novel derivatization reagent. *Techniques in Protein Chemistry IV*, 289 – 298 (R.H. Angeletil, Ed.) Academic Press ,1993, San Diego, CA, USA.