

Effect of endogenous methane production: a step forward in the validation of Biochemical Methane Potential (BMP) tests

João V. Oliveira^{1,2}, José C. Costa^{1,2}, Ana J. Cavaleiro^{1,2}, M. Alcina Pereira^{1,2}, M. Madalena Alves^{1,2,*}

1 CEB – Centre of Biological Engineering, University of Minho, 4710-057 Braga, Portugal;
jvoliveira@ceb.uminho.pt (JVO); acavaleiro@deb.uminho.pt (A.J.C.); alcina@deb.uminho.pt (M.A.P.);
madalena.alves@deb.uminho.pt (M.M.A.)

2 LABBELS – Associate Laboratory, Braga/Guimarães, Portugal

* Correspondence: madalena.alves@deb.uminho.pt (M.M.A.)

SUPPLEMENTARY MATERIALS

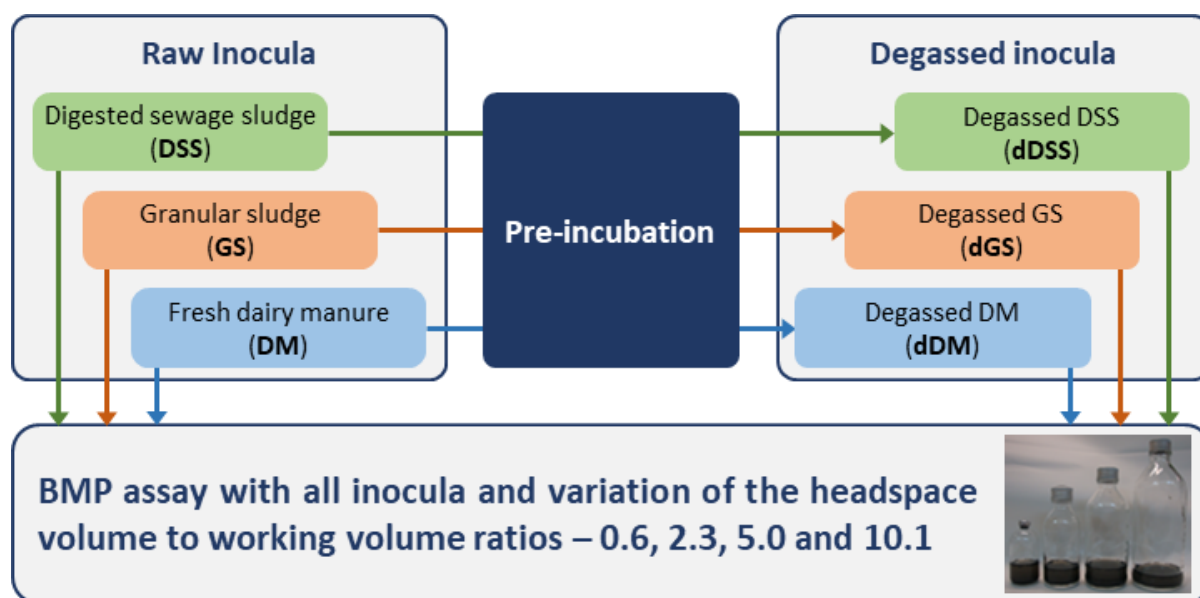


Figure S1. Experimental design of the inocula pre-incubation and BMP assays.

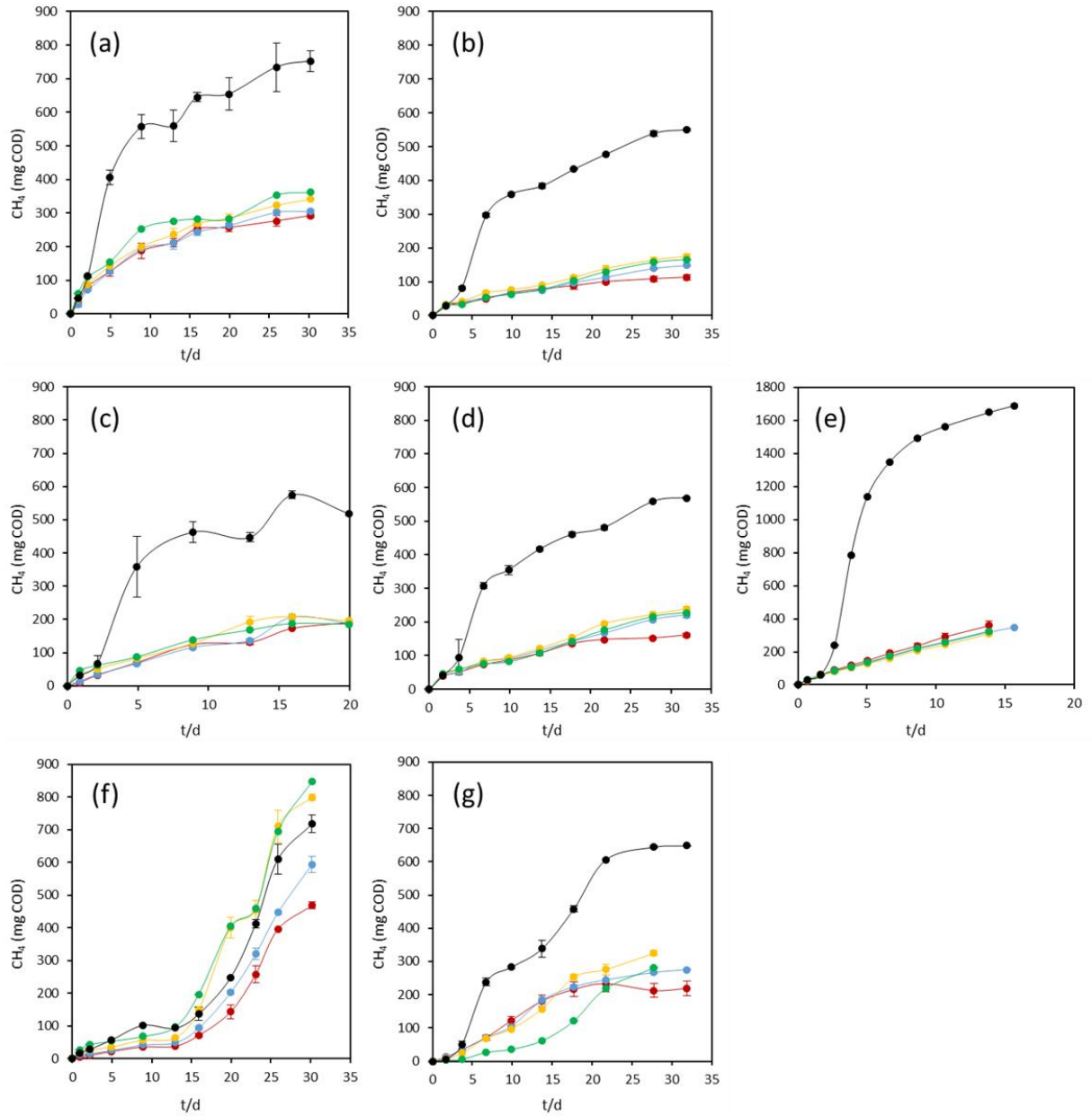


Figure S2. Cumulative methane production of cellulose (●) and in the blanks with different $\text{Hv} \cdot \text{Wv}^{-1} = 0.6$ (●), 2.3 (●), 0.5 (●), and 10.1 (●) – in the assays inoculated with (a) DSS; (b) dDSS; (c) GS; (d) dGS; (e) dGS-5d; (f) DM; and (g) dDM.

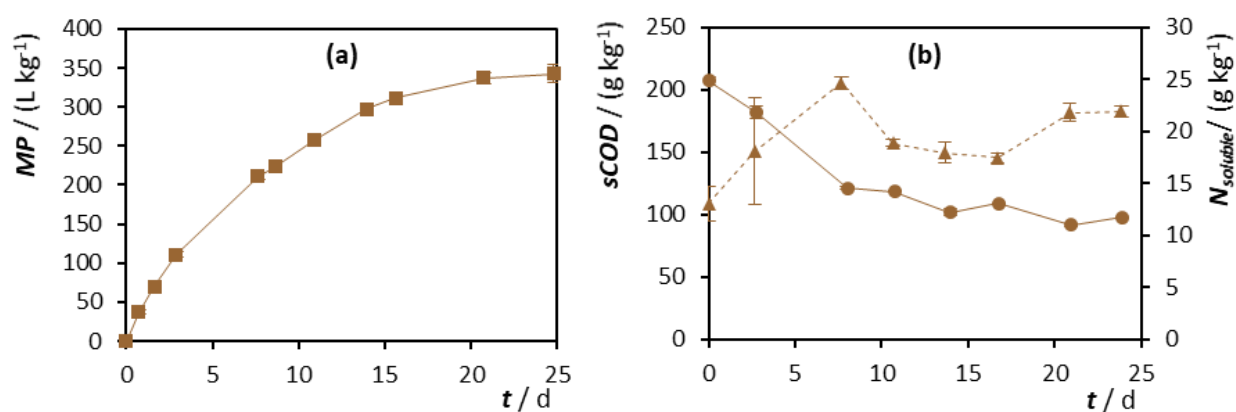


Figure S3. Key parameters measured during the degassing process of the mixture of DM and GS (50:50 % w/w): (a) MP (■); (b) sCOD (●) and $N_{soluble}$ (▲). All parameters are expressed per kg of VS of inoculum added at the beginning of the assay.

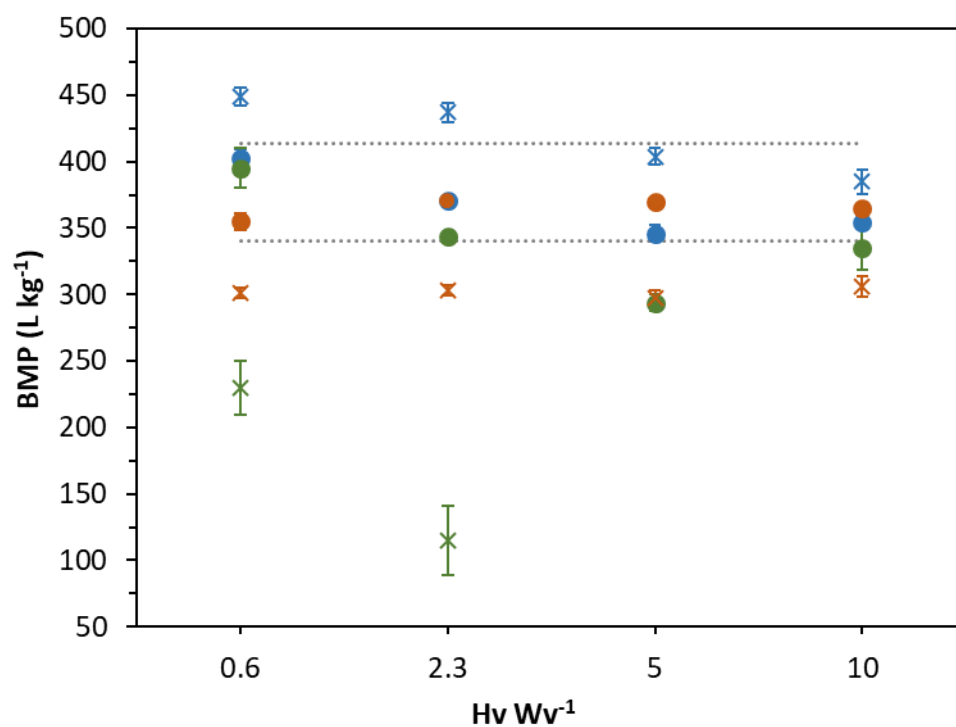


Figure S4. BMP values of cellulose obtained for the different inocula, after discounting the background methane production from the blank assays with the different Hv Wv⁻¹. DSS (x), dDSS (●), GS (x), dGS-5d (●), DM (x) and dDM (●). The dotted grey lines represent 82 % and 100 % of the theoretical BMP values (i.e., 340 L kg⁻¹ and 414 L kg⁻¹, respectively).

Table S1. Cumulative methane production (mL at STP conditions) at the end of the assays in the blanks with different $H_v \cdot W_v^{-1}$ (0.6, 2.3, 5.0 and 10.1), for the different inocula studied

Inoculum	Blanks with different $H_v \cdot W_v^{-1}$			
	0.6	2.3	5.0	10.1
DSS	102 ± 2	108 ± 1	120 ± 1	129 ± 1
dDSS	40 ± 3	52 ± 1	61 ± 3	58 ± 3
GS	99 ± 3	181 ± 2	68 ± 3	108 ± 2
dGS	64 ± 2	89 ± 2	101 ± 2	99 ± 0
dGS-5d	192 ± 13	168 ± 1	165 ± 1	178 ± 5
DM	164 ± 4	208 ± 9	280 ± 4	296 ± 4
dDM	77 ± 7	106 ± 0	127 ± 3	116 ± 5

Table S2. Methane (μmol) measured in the 0.5 mL samples collected from the bottles with the pressure lock syringe for GC analysis, at the end of the assays

Inoculum	Blanks with different $H_v \cdot W_v^{-1}$				Cellulose ^(*)
	0.6	2.3	5.0	10.1	
DSS	31.9 ± 0.7	9.6 ± 0.2	5.0 ± 0.0	2.6 ± 0.1	11.0 ± 0.4
dDSS	12.2 ± 0.9	4.7 ± 0.1	2.5 ± 0.1	1.2 ± 0.1	8.0 ± 0.1
dGS-5d	41.9 ± 3.2	11.7 ± 0.2	4.8 ± 0.1	2.5 ± 0.0	13.0 ± 0.1
dM	23.9 ± 2.3	8.7 ± 0.1	4.7 ± 0.1	2.0 ± 0.1	9.5 ± 0.0

(*) Theoretically, if all the added cellulose was degraded in the controls, sampling 0.5 mL at the end of the assay in these bottles would lead to the quantification of 12.8 and 7.7 μmol of methane in the assays with dGS-5d and all the other inocula, respectively.