Selected biochars can reduce methane production *in vitro*

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Biochars produced by pyrolysis of diverse waste biomass gained attention as rumen modifiers in recent years, in particular to promote microbial activity and reduce enteric methane (CH_4) production (Winders *et al.* 2019). As they can vary in parent material, pyrolysis temperature and post-pyrolysis alterations, they may also differ in the physical and chemical properties (Taherymoosavi *et al*. 2016). It may be possible to explore and exploit the diversity of biochar properties to engineer fit-for-purpose biochar to promote fermentation and efficiently and safely reduce CH_4. The objective of this study was to examine effect of biochars differing in parent substrates and production processes on total microbial gas (as indicator of microbial activity) and CH_4 production when combined with substrate and fermented *in vitro*.

Thirteen biochars (T1-T13) were selected having variable parent substrates, pyrolysis temperatures and additives - *Acacia cambagei* hardwood (T1-T5, 400°C), *Eucalyptus marginata* hardwood (T6 - T9, 600°C), mixed hardwood only (T11 - T12, 600°C), or combined with *Melaleuca alternifolia* mulch and soybean residue (T13, 400°C). Apart from biomass, the parent substrate in T1 and T12 also contained zeolite, while T10 and T11 had bentonite. The post-pyrolysis alterations involved acidification (T1, T2, T4, T11, T12), or addition of glycerol (T1, T6, T7, T10, T12), molasses (T3), and two biochars were aged for 1 year (T6, T8). The biochars were combined with oaten chaff as substrate and examined in triplicate in an *in vitro* batch assay, where total gas and CH_4 production were measured after termination of the 24 h incubation period. In the initial screening, all 13 biochars were tested at 20 g/100 g substrate. Three biochars with no reduction in gas, and/or reduction in CH_4 were selected and further examined at lower inclusion levels (1 g, 5 g and 10 g/100 g).

There was variability in total gas and CH_4 production between 13 biochar treatments, with T1 promoting gas production (by 1.5%), T12 reducing CH_4 by 5% without reducing gas, and T13 significantly ($P<0.05$) reducing CH_4 by 8%, but also reducing total gas (by 2%, Figure 1). When these three biochars were tested at lower levels, they all significantly ($P<0.05$) reduced CH_4 compared to treatment without biochar (0 g/100 g substrate), and the most effective were T1 at 10 g/100 g (34% reduction), and T12 and T13 at 5 g/100 g in (36% and 35% reduction, respectively), with only T12 at 5 g/100 g reducing CH_4 without reducing total gas production.

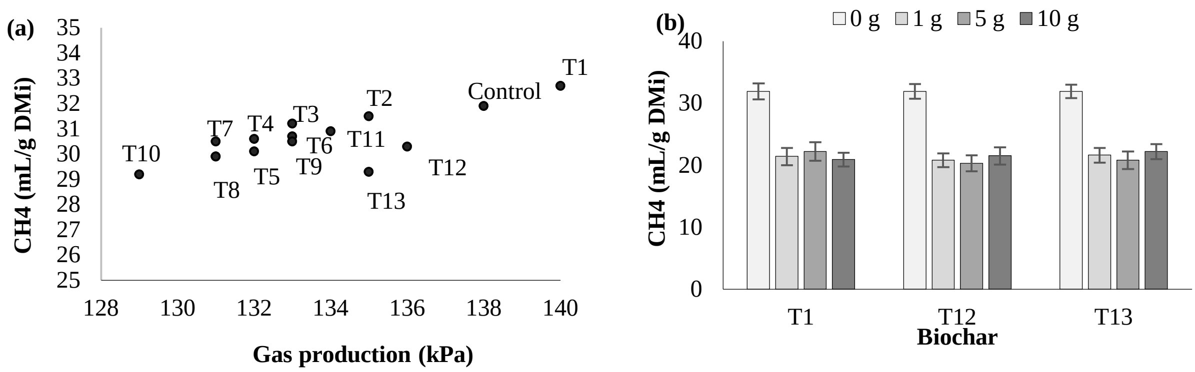

Figure 1. Total gas (kPa) and/or CH_4 production (mL/g dry matter incubated, DMi) from *in vitro* incubations with different biochars: (a) 14 biochars at 20 g/100 g substrate, (b) four biochars at three levels

We have demonstrated that biochars vary in their effect on rumen gas and CH_4 production, and the effect was linked to the type of biochar, as well as its inclusion level. Some of the biochars reduced CH_4 production *in vitro* by up to 36%, with no apparent effect on substrate digestibility. It is necessary to explore the possibility of rumen adaptation to fit-for-purpose biochar in extended studies *in vivo*.

References

Taherymoosavi, S, Joseph S and Munroe P (2016) *Journal of Analytical and Applied Pyrolysis*. **120**, 441-449.

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