

# **Inhibition of methane production through anaerobic digestion by Preventol**

## **Experimental protocol**

The automatic methane potential test system (AMPTS) nowadays routinely employed in evaluating the biodegradability or biochemical methane potential of a substrate [1-2] was used as an experimental tool in the present assay. The application of the AMPTS and experimental protocol have been well documented [1].

To evaluate the effect of a specially prepared solution of preventol (2-Bromo-2-nitropropane-1,3-diol) on methane generation from microcrystalline cellulose, five different tests, all in triplicates under mesophilic conditions were adopted and evaluated with the aid of the AMPTS. The tests were;

- 1) Addition of 1 ml of preventol solution to 300 ml of pre-incubated inoculum, to which 2.3 g of pre-dried cellulose was added as substrate.
- 2) Same as in '1' with the addition of 40  $\mu$ l of preventol instead.
- 3) Same as in '1' with the exception that preventol was not added, this also acted as a positive control.
- 4) Consisted of 300 ml of inoculum only, serving as negative control.
- 5) Was same as in '4' with the addition of 1 ml preventol solution.

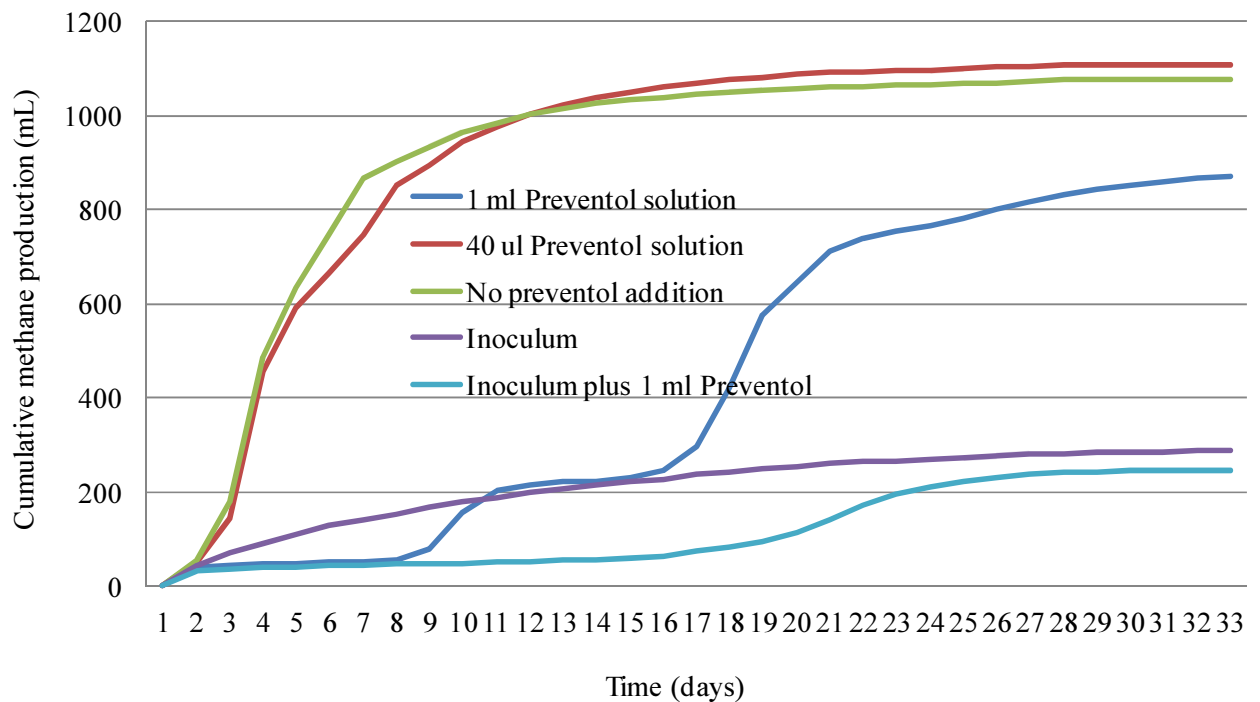
## **Results**

Figure 1 shows the cumulative methane production from all tests with and without preventol addition. There was no noticeable difference between methane production from cellulose without addition of preventol and addition of 40  $\mu$ l of preventol solution.

On the other hand, addition of 1 ml preventol solution resulted in a lag phase for up to eight days. Methane production from the 1 ml preventol test with cellulose showed a two-stage (diauxic) methane production curve with production levelling up on day 11 and commencing again on day 16. This result is in line with findings that microbes in the presence of 2-bromo-2-nitropropane-1,3-diol indicate a period of biocide-induced bacteriostasis followed by growth at an inhibited rate [3].

Therefore, the 32-day methane production was significantly lower than the ones from the tests without preventol addition and addition of 40  $\mu$ l.

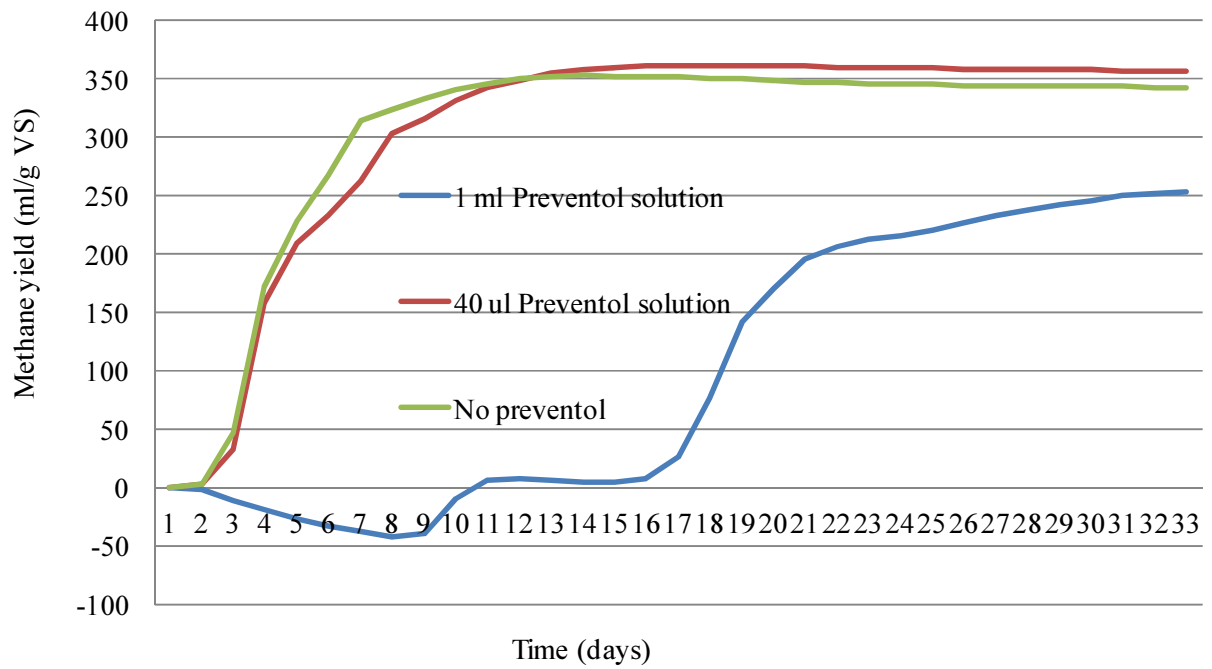
Addition of 1 ml of preventol solution to the inoculum only also led to an extended lag phase with production commencing on day 23. However, the methane production from inoculums with and without preventol addition was comparable after 32 days of incubation.



**Figure 1.** Cumulative methane production from microcrystalline cellulose and inoculums with and with addition of preventol.

It is plausible to state therefore that the concentration of the bacteriocidal or bacteriostatic ingredient in 40  $\mu$ l of preventol was not enough to elicit an inhibition of the anaerobic microorganism involve in methane production. On the other hand, 1 ml preventol solution could lead to a partial inhibition of the anaerobes.

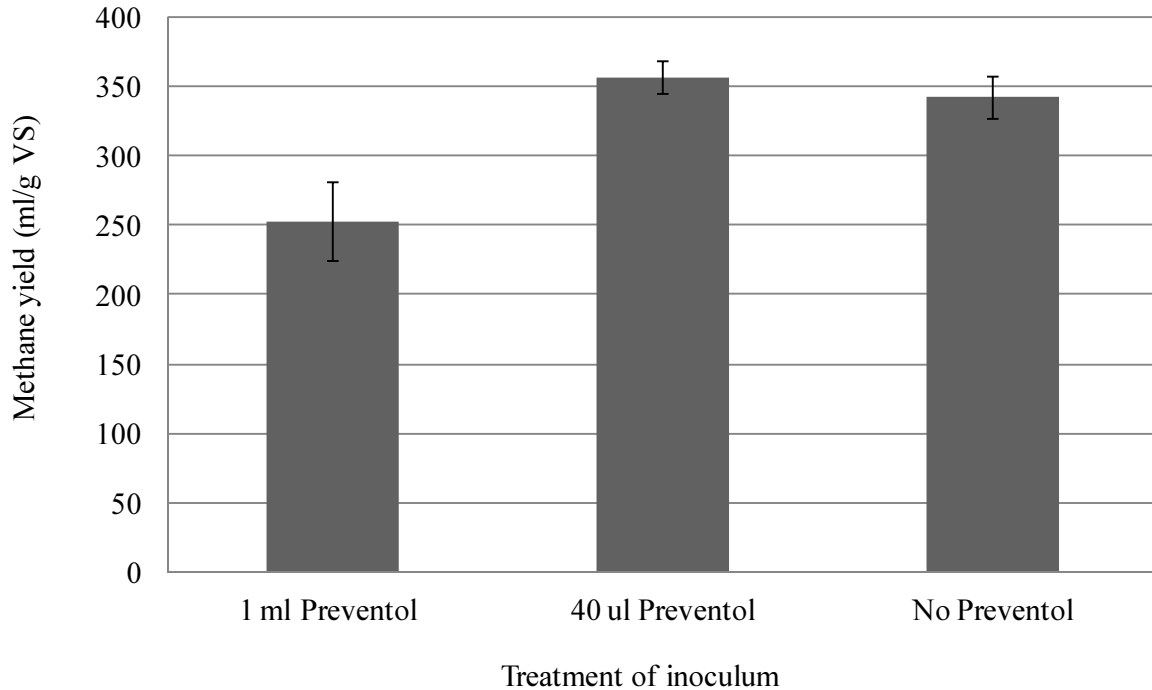
Figure 2 and 3 shows the cumulative and ultimate methane yields of cellulose after 32 days of incubation. The negative methane yield noted for the test with 1 ml preventol addition during the first ten days of incubation was because the methane production from the inoculum or background was higher (Figure 1).



**Figure 2.** Cumulative methane yield of microcellulose with and without addition of preventol.

The ultimate methane yield of the positive control (no preventol addition) and 40  $\mu$ l preventol addition were not significantly different. In fact, the yields are in the same range as experimental methane yields of cellulose reported in literature [2, 4] Contrarily, the yield from the 1 ml preventol test was significantly lower.

The yields achieved in this study were on average 60.1%, 85.7% and 82.7% of the theoretical methane yield of cellulose for 1 ml, 40  $\mu$ l and no preventol addition respectively.



**Figure 3.** Ultimate methane yields of cellulose with and without addition of preventol.

The inhibitory mechanism of preventol on the anaerobic microorganisms is not clear. However, one can liken the inhibition to that of another bromo-molecular called 2-bromoethane sulfonic acid (BESA). BESA, which is a closely related analog of Coenzyme M or CoM (2-mercaptoethane sulfonic acid) is known to inactivate methanogenesis through the inhibition of the methylreductase-F<sub>430</sub> enzyme complex [5]. This premise may be supported by another thesis which state that the antimicrobial activity of preventol relates to the interaction with essential thiols (e.g. CoM and HS-CoM which is formed during the reduction of the methyl by hydrogen) within the cell [3]

## References

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