Biochemical methane potential of waste activated sludge: Influence of inoculum pre-incubation

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The biochemical methane potential (BMP) test is a method used to determine the methane potential and biodegradability of organic wastes, such as sewage sludge, in anaerobic digestion batch reactors. The BMP test requires detailed configuration of various conditions. However, there is no single standard method that is universally used and only a number of guidelines are available (Himanshu et al., 2017). Pre-incubation (starvation) is necessary to deplete the remaining biodegradable organic material in the inoculum to reduce error when BMP testing. The duration of pre-incubation is typically 2–5 days, although some studies have reported as little as 1 day and as much as 14 days (Filer et al., 2019; Hulsemann et al., 2020). However, it may be necessary to starve the inoculum for a longer period to minimize biogas generation, depending on the type of wastewater/waste and reactor conditions (Angelidaki et al., 2009). It is difficult to present a criterion for pre-incubation in terms of duration. Therefore, this study aims to address this issue by presenting a quantitative criterion for pre-incubation based on the effective volume of the reactor and daily biogas production.

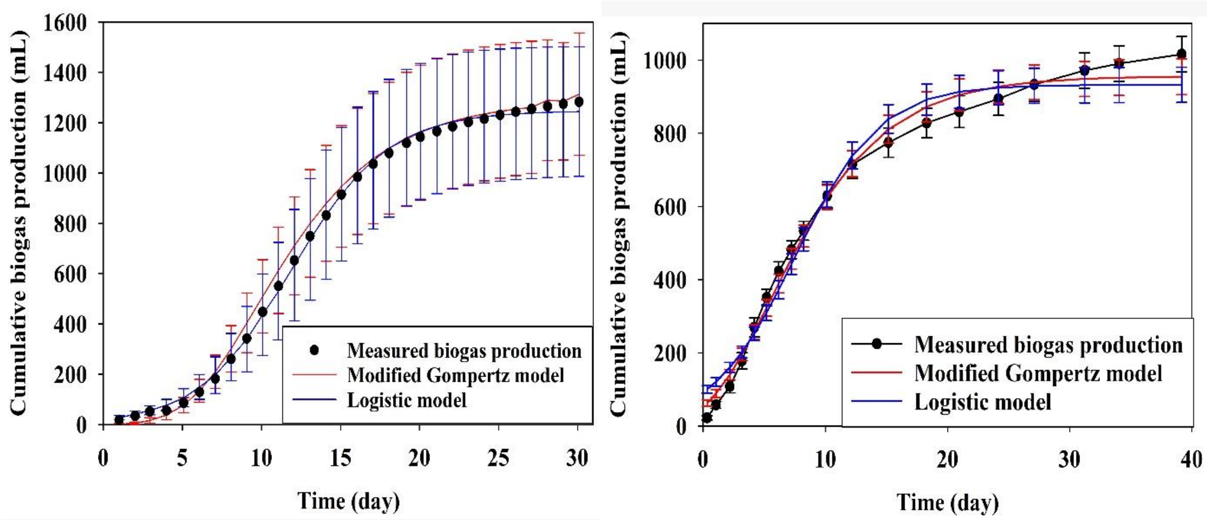
The experiment involved running 28 reactors with a total volume of 500 mL and an effective volume of 285 mL for pre-incubation. The inoculum's volatile fatty acids (VFA) composition consisted of 4,590 mg/L of acetic acid, 391 mg/L of propionic acid, 124 mg/L of isobutyric acid, 465 mg/L of butyric acid, 231 mg/L of isovaleric acid, 48 mg/L of valeric acid, and 48 mg/L of caproic acid, using digested sludge with high VFA concentration. The BMP test involved operating six reactors under identical conditions with a 1:1 ratio of starved inoculum to waste activated sludge in terms of volatile solids (VS). Kinetic analysis was conducted using the modified Gompertz and Logistic models.

The digested sludge composition was compared after pre-incubation, revealing a removal of 18% of total solids (TS) and 27% of VS. The solubilization rate (soluble chemical oxygen demand (SCOD)/total chemical oxygen demand (TCOD)) decreased by 18%. The inoculum had high levels of VFAs, but only acetic acid (51 mg/L) was detected after pre-incubation. This effectively removed the high accumulation of VFAs in the substrate, which can be a source of error in BMP testing. During the pre-incubation period, the cumulative biogas production was 1,293±268 mL (coefficient of variation (CV): 21%) after 30 days. The daily biogas production decreased to 8.2±1.4 mL, eventually falling below 10 mL. Before pre-incubation, the dominant bacterial phylum was Bacteroidota (17.7%), Pseudomonadota (15.8%), and Bacillota (14.3%). After pre-incubation, the dominant phylum was Pseudomonadota (21.8%), Bacillota (11.5%), and Bacteroidota (9.1%). Bacteroidota, which are microorganisms that break down and ferment polysaccharides into monosaccharides (Vanwonterghem et al., 2014), decreased by 8.6% after pre-incubation, which is likely due to the removal of organic material through pre-incubation. Bacillota is mainly found in the acidogenic phase. The 2.8% decrease in Bacillota after pre-incubation is likely due to the conversion of VFAs to methane through pre-incubation. Before pre-incubation, the dominant archaeal families were Methanoregulaceae (39.2%), Methanotrichaceae (23.3%), and Methanomicrobiaceae (17.5%). After pre-incubation, the dominant archaeal families were Methanoregulaceae (34.5%), Methanotrichaceae (34.1%), and Methanomicrobiaceae (15.6%). The decrease in the share of Methanoregulaceae is due to their energy acquisition process of reducing carbon dioxide to methane. After pre-incubation, Methanotrichaceae increased by 10.8%. This is likely due to their relatively high proportion when VFA is below 0.5 g/L (Maria, 2017).

In the BMP test with starved inoculum, VS removal was 42.8±2.2% (CV: 5.1%) and SCOD removal was 87.8±1.6% (CV: 1.8%) with a small CV. In the case of VFA, only acetic acid (11.0±2.6 mg/L) was detected, and the alkalinity was 4,020±128 mg/L, indicating that the BMP test was stable. The cumulative biogas production was 1,030±40 mL (CV: 3.9%). The cumulative methane production was 611±22 mL (CV: 4.0%). The biogas yield was 654±31 mL/VS added (CV: 4.7%), and the methane yield was 389±19 mL/VS added (CV: 4.8%). These results confirm the stability of the BMP test using the starved inoculum (Figure 1).

The predicted biogas production was 1,293±254 mL (CV: 19.7%), and the maximum biogas production rate was 167±34 mL/day (CV: 31.9%) with modified Gompertz model for the pre-incubation. The predicted biogas production was 1,248±256 mL (CV: 20.5%), and the maximum biogas production rate was 110±33.8 mL/day (CV: 30.7%) with the logistic model for the pre-incubation. The CV for predicted biogas production was 5.2%, and for maximum biogas production rate it was 5.3% with modified Gompertz model for the BMP test. The predicted biogas production had a CV of 5.2%, and the maximum biogas production rate had a CV of 5.4% with the logistic model for the BMP test (Figure 1). The CVs of predicted biogas production and maximum biogas production rate in the BMP test were lower than those in pre-incubation.

For a reactor with an effective volume of 285 mL, analysis of the digested sludge at the point when daily biogas production was reduced to < 10 mL showed a 99.2% decrease in volatile fatty acids (VFA). There was a decrease in Bacteroidota and an increase in Methanotrichaceae. The CV for cumulative biogas production during pre-incubation was 21%. However, in the BMP test, the CV decreased to 3.9%, indicating a reduction of 17.1%. The CV for the maximum biogas production rate in kinetic analysis also decreased by 26.6%. Therefore, we propose a standard criterion of less than 0.035 $mL_{biogas}/mL_{working\ volume}$/day for pre-incubation in the BMP test.



(a) pre-incubation (b) biochemical methane potential test

Figure 1. Measured and predicted cumulative biogas production using modified Gompertz and Logistic models in (a) pre-incubation and (b) biochemical methane potential test.

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