



# The effect of substrate to inoculum ratios on the anaerobic digestion of human faecal material



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## HIGHLIGHTS

- The anaerobic digestion of human faecal material was investigated.
- The substrate to inoculum ratio (SIR) was important for methanogenesis.
- The highest amounts of methane production was achieved in the 0.5 SIR incubations.
- The highest pathogen removal was also achieved in the 0.5 SIR incubations.

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## ABSTRACT

The anaerobic digestion (AD) of human faecal material (HFM) was investigated to consider the effect different substrate to inoculum ratios (SIR) from 0.5 to 4 on the rate and extent of methane production as well as impact on pathogen numbers. The AD process was monitored by measuring pH, total volatile fatty acid, bicarbonate alkalinity, ammonium and methane production. The results showed that the highest amounts of methane production with a value of  $254.4 \pm 12.6 \text{ ml CH}_4 \text{ g VS}_{\text{added}}^{-1}$  and highest pathogen removal with a value of  $2.7 \times 10^4 \pm 40$  and  $2.5 \times 10^3 \pm 0.5 \text{ CFU/ml}$ , respectively, for *E.coli* and faecal coliform bacteria was achieved by the 0.5 SIR incubation. However, the highest organic loading found in the 4.0 SIR incubation showed the lowest methane yield with a value of  $110 \pm 1.3 \text{ ml CH}_4 \text{ g VS}_{\text{added}}^{-1}$  and the lowest pathogen removal with a value of  $3.2 \times 10^5 \pm 19$  and  $3.2 \times 10^4 \pm 3.5 \text{ CFU/ml}$ , respectively for *E.coli* and faecal coliform bacteria. The empirical equation was used to calculate the theoretical methane and compare this with the actual values of methane production. The relatively high methane conversion efficiency between theoretical and actual values for 0.5 SIR, further suggest that this operational condition was the most effective.

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## 1. Introduction

There are over 7 billion people in the world with a faecal output of 1–2 kg/person/day, equating to 7–14 million tonnes of faecal waste generated on a daily basis (Bloom, 2011; Wyman et al., 1978). This is an enormous amount of energy that could be generated if the human faecal material (HFM) was used as a feedstock for driving anaerobic digestion (AD). In the UK, the centralised sewage collection and treatment facilities are used to manage HFM; however, decentralised sewage collection systems are more common in America, Asia and sub-Saharan Africa in which the use of septic tanks are more common (EPA, 2010; Jewitt, 2011). However, the introduction of septic tanks in Asia and Africa has not been successful for treating HFM (Esrey et al., 1998; Jewitt, 2011). This is due to the continual seepage of faecal effluent into water bodies, which contributes to the destruction of aquatic ecosystems and the proliferation of water borne diseases, such as dysentery and cholera (Esrey et al., 1998; Park et al., 2001).

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More recently, the use of HFM as a feedstock for AD has continued to increase, particularly in developing countries (Katukiza et al., 2012). For example, in India, some latrines are being converted into anaerobic digesters, for biogas production; however, the effluents have been reported to contain high levels of pathogenic bacteria, such as *Salmonella* and *Shigella* (Kunte et al., 2000). According to Kunte et al. (2000), the health risk involved in handling HFM has deterred most operators from considering this material as a feedstock for AD. Additionally, the ineffectiveness of AD to reduce the numbers of pathogens to an acceptable level, as described in PAS 110, could represent an additional concern (Astals et al., 2012). To combat this, Astals et al. (2012) reported that a pre-treatment stage in the AD of HFM would further decrease the pathogen load. Another major concern is the AD of HFM is the low levels of methane production. For example, Rajagopal et al. (2013) reported that the extent of methane production from HFM (260–300 ml CH<sub>4</sub> g VS<sub>added</sub><sup>-1</sup>) was lower than that of food waste (400–420 ml CH<sub>4</sub> g VS<sub>added</sub><sup>-1</sup>). A relatively new development is to co-digest HFM with a high energy substrate, such as glycerol or lignocelluloses, because the nutrient content of HFM is comparatively high, resulting in greater amounts of methane production (Zhang et al., 2012).

HFM is considered as the most readily available resource for AD (Katukiza et al., 2012). The utilisation of HFM as a potential feedstock for AD in small settlements, military barracks, refugee camps, school, and other institutions holds massive potential as a renewable energy source. For most developing countries, this can help to reduce the reliance on trees for firewood, as well as on industrially produced fertiliser (Arthur et al., 2011; Nzila et al., 2012). However, the presence of appreciable numbers of pathogenic bacteria in the digestate has to be considered. Therefore, the aim of this study was to evaluate the effect of substrate to inoculum ratios (SIRs) and nutrient content of HFM on the process performance and pathogen content during anaerobic digestion. The process performance was assessed by measuring methane production, initial and residual total volatile fatty acid concentrations, ammonium levels and organic matter removal. To assess the impact of SIR on pathogen numbers, *E.coli* and faecal material were also measured.

## 2. Materials and methods

### 2.1. Materials

#### 2.1.1. Substrate

The fresh human faecal samples used in this study were collected in a specially designed and installed sole-segregated toilet. The samples were collected daily for 7 d, mixed and stored in the refrigerator at 4 °C prior to use. The physicochemical characteristics of the sample are represented in Table 1.

#### 2.1.2. Inoculum

The digested sewage sludge-inoculum was collected from the domestic and industrial wastewater treatment plant in Lancaster, UK. The AD treatment plant is operated at mesophilic temperature (36 °C) and is used to treat secondary sludge. Prior to storage and chemical analysis of the inoculum, suspended particles and debris were removed by passing it through 0.75 mm sieve. The digested sewage sludge-inoculum was stored in a refrigerator for a maximum of 3 d at 4 °C prior to the experiment. The inoculum was characterised and contained 1.196% w/w volatile solids (VS), 1.93% total solids (TS).

**Table 1**  
Physicochemical characterisation of the substrates.<sup>a</sup>

Parameter	Faecal material
Protein (g/kg)	6.10
Carbohydrate (g/kg)	41.90
Carbon (%)	47.70
Nitrogen (%)	6.12
C/N	7.79
Total solid (w/w%)	23.75
Volatile solid (w/w%)	21.05
NH <sub>4</sub> -N (g/l)	1.49
MPN ( <i>E. coli</i> ) CFU/ml	8.6 × 10 <sup>8</sup>
MPN (faecal coliform bacteria) CFU/ml	4.8 × 10 <sup>7</sup>
pH	7.36

<sup>a</sup> All values were within 5% of expected value.

### 2.2. Methods

#### 2.2.1. Anaerobic digestion

This experimental study was carried out using a modified batch reactor that comprised of a Duran bottle with an operational volume of 500 ml. The reactors were continuously stirred with 12 V DC motors (Lojer component, UK) at 30 rpm and placed in a digital thermostatic water bath at a mesophilic temperature (35 °C). The experimental set up was carried out in triplicate for 25 d. Each of the reactors was flushed with nitrogen gas at for a period of 30 s to remove oxygen before tightly inserting the lid. The SIRs of 4.0, 3.0, 2.0, 1.0, 0.5 and 0.0 were maintained for the batch reactors. A constant inoculum mass of 2.29 g VS and an increasing mass of substrate ranging from 0.0 to 9.18 g VS was maintained (Table 2).

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