Microbiological Research 167 (2012) 630–636

Contents lists available at SciVerse ScienceDirect

Microbiological Research

journal homepage: www.elsevier.de/micres

Effects of different nitrogen sources on the biogas production – a lab-scale investigation

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ARTICLE INFO

Article history:
Received 13 September 2011
Received in revised form
15 November 2011
Accepted 26 November 2011

Keywords:
Nitrogen source
Anaerobic digestion
Nitrogen
Biogas
Methane

ABSTRACT

For anaerobic digestion processes nitrogen sources are poorly investigated although they are known as possible process limiting factors (in the hydrolysis phase) but also as a source for fermentations for subsequent methane production by methanogenic archaea. In the present study different complex and defined nitrogen sources were investigated in a lab-scale experiment in order to study their potential to build up methane. The outcome of the study can be summarised as follows: from complex nitrogen sources yeast extract and casamino acids showed the highest methane production with approximately 600 ml methane per mole of nitrogen, whereas by the use of skim milk no methane production could be observed. From defined nitrogen sources L-arginine showed the highest methane production with almost 1400 ml methane per mole of nitrogen. Moreover it could be demonstrated that the carbon content and therefore C/N-ratio has only minor influence for the methane production from the used substrates.

1. Introduction

Anaerobic digestion has become very popular in recent years and therefore instead of traditional aerobic composting an increasing ratio of biowaste is nowadays treated anaerobically in closed systems. The main advantage of anaerobic digestion is the production of energetically utilisable biogas (methane) (Schink 2002). Next to this unpleasant odours are minimised during fermentation and most pathogens do not survive high fermentation temperatures (thermophilic digestion) and/or matrix composition. The effect of many different carbon sources on the microbial decomposition process and methane production in anaerobic digestion systems was previously investigated in various studies (e.g. Wagner et al. 2010, 2011), whereas nitrogen sources are poorly investigated although they are known as possible process limiting factors (Scherer 2001) but also as a source for fermentations for subsequent methane production by methanogenic archaea (Gottschalk 1986). Nitrogen reaches the anaerobic digestion process mainly in form of proteins, amino acids, and urea in order to be further degraded (Fang et al. 1994; Angelidaki and Ahring 1993; Yokoyama et al. 2007). Amino acids obtained from proteins by degradation via extracellular proteases and purine- and pyrimidine-bases are fermented by a variety of microorganisms (e.g. alanine – *Clostridium propionicum*, glycine – *Peptostreptococcus micros* or glutamate – *Clostridium tetanomorphum*). Although some amino acids can be used as sole carbon/nitrogen sources, Clostridia mainly prefer to ferment amino acids in pairs within the so-called Stickland-reaction. Often the end- or by-product of the decomposition process is NH_3/NH_4^+, which can be used by various microorganisms directly as a nitrogen source and which additionally affects the buffer system of surrounding media (Chen et al. 2008). In liquid phase NH_3 and NH_4^+ are at permanent equilibrium ($NH_4^+ \leftrightarrow NH_3 + H^+$ and $NH_4^+ + OH^- \leftrightarrow NH_3 + H_2O$) (Sung and Liu 2003) which is influenced by pH and temperature (Scherer 2001). In anaerobic digestion systems it may have a negative effect on various processes as described, for example, by Calli et al. (2005). Especially methanoarchaea are known to be more sensitive against NH_3 stress compared to bacteria (Heinrichs et al. 1990; Hansen et al. 1998; Sawayama et al. 2004; Fricke et al. 2007). However, the sensitivity of the two methanogen groups – hydrogenotrophic and acetoclastic methanogens – is discussed controversially, but compared to hydrogenotrophic methanogens acetoclastic methanogens show a higher sensitivity against NH_3 (Wiegant and Zeeman 1986; Borja et al. 1996; Gallert and Winter 1997).

In the present study different complex and defined nitrogen sources have been added in amounts to reach a constant nitrogen concentration of 120 mM and their effect on the anaerobic methane building process has been examined.

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doi:10.1016/j.micres.2011.11.007

2. Material and methods

2.1. Samples and sampling

Sludge samples were taken from the outlet sampling port of a 750,000-litre anaerobic digestion plant (Biogaskraftwerk) in Roppen, Austria, described by Illmer and Gstraunthaler (2009). Sample volumes of approximately one litre were taken, aliquoted, and used for subsequent analysis. For characteristic fermenter running conditions and some overall performance parameters please refer to Illmer and Gstraunthaler (2009) and Wagner et al. (2009a). Considerations on the microbial community running the digestion processes in the biogas plant can be found in Malin and Illmer (2008). There, the microbial digester community could be shown to be quite inured to process disruptions and contradictory to the opinion that the main part (up to 2/3) of methane is derived from acetoclastic methanogenesis (Gerardi, 2003) no members of this group have been found in relevant amounts. The analysis of the microbial population in the inoculum revealed *Methanoculleus* sp., *Methanothermobacter wolfeii*, and an uncultured bacterium showing slight sequence homology with *Geobacillus* sp. as the most abundant microorganisms after molecular biological analysis.

2.2. Inoculum preparation

The inoculum was prepared as follows (according to Wagner et al. 2010): still hot, autoclaved distilled water was flushed for 10 min with gas consisting of 70% N_2 and 30% CO_2 during cooling process in order to ensure oxygen free conditions. Subsequently, approximately 100–150 g of sludge were added to obtain a dilution of 1:5, before the flask was flushed again and closed with a butyl rubber septum (Ochs, Germany). The flask containing the diluted fermenter sludge (DFS) was shaken at 200 rpm for 30 min at 52 °C. Inoculum prepared in this way was aliquoted and frozen (−20 °C) until use.

2.3. Media preparation

Serum flasks (with a total volume of 120 ml) were either filled with 50 ml (including a nitrogen source) or 45 ml (adding the nitrogen source form a stock solution) of minimal medium (modified according to Widdel (1980)) containing (per litre distilled water): 1.0 g NaCl, 0.4 g $MgCl_2{\cdot}6H_2O$, 0.2 g KH_2PO_4, 0.25 g NH_4Cl, 0.5 g KCl, 0.15 g $CaCl_2{\cdot}2H_2O$, 0.5 g L-cysteine, 2 ml Na_2S solution (60 g Na_2S l^{-1} distilled water), 1 ml vitamin solution (10 mg cyanocobalamine, 10 mg p-aminobenzoic acid, 2 mg D(+)-biotin, 20 mg nicotinic acid, 5 mg Ca–D(+)-pantothenate, 50 mg pyridoxamine–dihydrochloride, and 36 mg thiamine–dihydrochloride dissolved in 200 ml distilled water), 1 ml Resazurin solution as redox indicator (11.5 mg 10 ml^{-1} distilled water), 1 ml trace element solution (1500 mg $FeCl_2{\cdot}2H_2O$, 70 mg $ZnCl_2$, 100 mg $MnCl_2{\cdot}4H_2O$, 190 mg $CoCl_2{\cdot}6H_2O$, 2 mg $CuCl_2{\cdot}2H_2O$, 24 mg $NiCl_2{\cdot}6H_2O$, 36 mg $Na_2MoO_4{\cdot}2H_2O$, 6 mg H_3BO_3, 10 ml 25%–HCl, 1000 ml distilled water), and 1 ml Se–W solution (0.5 g NaOH, 3 mg $Na_2SeO_3{\cdot}5H_2O$, 4 mg $Na_2WO_4{\cdot}2H_2O$, 1000 ml distilled water). The flasks were filled using an anaerobic filling and cultivation vessel (Ochs, Germany); the headspace was flushed with gas (70% nitrogen, 30% CO_2) to remove oxygen, closed with butyl rubber septa, and sealed with aluminium caps.

Nitrogen sources were either added to the flask from a concentrated stock solution (1200 mM nitrogen), or (if not soluble enough) directly to the medium to achieve a final concentration of 120 mM nitrogen in a final volume of 50 ml in the respective flask.

Table 1
Nitrogen and carbon content of the used chemicals as determined by CHNS analysis and manufacturers declaration.

Nitrogen source	MW [g mol^{-1}]	Carbon [%]	Nitrogen [%]	C/N ratio
CAA	nd	32.4	8.0	4.05
Cas	nd	50.1	13.4	3.74
PfC	nd	44.0	12.6	3.49
YE	nd	40.9	10.5	3.90
PfS	nd	40.6	9.3	4.37
ME	nd	43.9	11.5	3.82
SM	nd	40.1	5.2	7.71
Gel	nd	45.0	15.0	3.00
PfG	nd	45.8	14.5	3.16
Arg	174.2	41.4	32.2	1.29
Lys	182.6	39.5	15.3	2.58
Ala	89.1	40.4	15.7	2.57
Met	149.2	40.3	9.4	4.29
His	155.2	46.4	27.1	1.71

CAA, casamino acids; Cas, casein; Gel, gelatine; ME, meat extract; PfC, peptone from casein; PfG, peptone from gelatine; PfS, peptone from soy; SM, skim milk; YE, yeast extract; Ala, L-alanine; Arg, L-arginine; His, L-histidine; Lys, L-lysine monohydrochloride; Met, L-methionine; nd, not determined.

2.4. Experimental setup and procedure

The study was conducted with different complex and defined nitrogen sources. Following complex sources were added in an amount to obtain a nitrogen concentration of 120 mM in the media: yeast extract (Merck), casein (Sigma), casamino acids (Difco), peptone from casein (Merck), peptone from soy (Merck), peptone from gelatine (Merck), meat extract (Merck), skim milk (Oxoid), gelatine (Merck); and as defined nitrogen sources: L-arginine (Merck), L-lysine monohydrochloride (Simga–Aldrich), D-alanine (Sigma–Aldrich), L-methionine (Sigma–Aldrich), and L-histidine (Sigma–Aldrich). Nitrogen and carbon content was determined on a LECO TruSpec analyser (CHNS analyser) (Leco, Germany) after manufacturer's declaration (Table 1).

All flasks containing the medium and the respective nitrogen source were inoculated with DFS in triplicate to reach a final concentration of 10%. Afterwards the flasks were incubated at 52 °C until the methane production ceased. Overpressure and gas composition were measured and gas production rates for H_2, CO_2, and CH_4 were calculated.

2.5. Analytics

For analyses of short chain fatty acids (SCFA) concentration 0.5 ml of the liquid were taken out via a syringe after intense shaking by hand for 5 s on day 0 and at the end of the investigation period. The samples were centrifuged at 20,000 g, filtered through a 0.2 μm RC filter, and injected (20 μl) directly into the HPLC system. If samples were not used for SCFA analysis immediately they have been deep frozen (−20 °C) until analysis day. The concentrations of SCFAs in the liquid phase (formic-, acetic-, propionic-, and butyric-acid) were measured via HPLC analysis on a BioRad AMINEX HPX-87H column, an isocratic system with 5 mM H_2SO_4, 65 °C, and a flow rate of 0.7 ml min^{-1}. A Shimadzu Prominence HPLC equipped with an UV-detector at a wavelength of 210 nm was used.

Pressure in the headspace resulting from degradation process was measured with a pressure sensor (Greisinger, Germany). The measurement of the pressure allowed the calculation of the volume of the total produced gases. Overpressure resulting form the increase in temperature at the beginning of the incubation and after each gas measurement was drained with a cannula after the liquid had reached the incubation temperature of 52 °C.

Analysis of CH_4, H_2, and CO_2 in the headspace was carried out using a Shimadzu GC2010 with a Wide Bore Injector (WBI)