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Syringe test screening of microbial gas production activity: Cases denitrification and biogas formation

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ABSTRACT

Mass produced plastic syringes may be applied as vessels for cheap, simple and large scale batch culture testing. As illustrated for the cases of denitrification and of biogas formation, metabolic activity was monitored by direct reading of the piston movement due to the gas volume formed. Pressure buildup due to friction was shown to be moderate. A piston pull and slide back routine can be applied before recording gas volume to minimize experimental errors due to friction. Inoculum handling and activity may be conveniently standardized as illustrated by applying biofilm carriers. A robust set of positive as well as negative controls ("blanks") should be included to ensure quality of the actual testing.

The denitrification test showed saturation response at increasing amounts of inoculum in the form of adapted moving bed biofilm reactor (MBBR) carriers, with well correlated nitrate consumption *vs.* gas volume formed. As shown, the denitrification test efficiently screened different inocula at standardized substrates. Also, different substrates were successfully screened and compared at standardized inocula.

The biogas potential test showed efficient screening of different substrates with effects of relative amounts of carbohydrate, protein, fat. A second case with CO_2 capture reclaimer waste as substrate demonstrated successful use of co-feeding to support waste treatment and how temperature effects on kinetics and stoichiometry can be observed.

In total, syringe test screening of microbial gas production seems highly efficient at a low cost when properly applied.

1. Introduction

In a time of increasingly complex and sophisticated methodology, it may be revealing to find fields of science where simplicity may still be relevant. Mass screening of microbial activity in open system batch cultures is one case still of significance. In principle, this is either applied to compare different substrates at a hopefully standardized inoculum, or to compare different inocula at some standardized substrate.

This is applied both in routine surveillance as well as in research, as in the OECD/ISO standardized tests for ecotoxicology and biodegradation (OECD, 1981, 1992, 2004, 2006a, 2006b). Either, some substrate consumption or some product formation may be recorded. Substrates in the widest sense could be some organic or inorganic electron donor, just as well as some acceptor as in the case of the respiratory BOD test (Clescerl et al., 1999). Product formation is only applicable for activity measurements in cases of product accumulation, mainly in cases of anaerobic or fermentative conditions.

In particular cases, microbes may create products forming a stable and separate gas phase, such as in the case of biogas production, given in Eq. (1):

$$\text{Organic matter} \rightarrow CH_4 + CO_2 \quad (1)$$

The exact product balance depends on the redox state of the substrate and the products' water solubility.

Another case is the type of anaerobic respiration denoted anoxic or denitrifying reaction, with NO_3^- as the electron acceptor, given in Eq. (2):

$$\text{Organic matter} + NO_3^- \rightarrow CO_2 + N_2 \quad (2)$$

The accumulation of inert dinitrogen gas will directly reflect the respiration rate. Due to its low water solubility, it will readily separate out as a gas phase.

So far, denitrification rates are estimated by sampling and repetitive chemical analysis of remaining nitrate as a function of time (Henry et al., 2016a). We are not aware of any direct gas production measurements.

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In the case of anaerobic fermentation, the headspace volume may be sampled for gas analysis. The OECD anaerobic test no. 311 is based on recording the increase in headspace pressure due to biogas formation at constant volume, involving rather complicated equipment and procedures (OECD, 2006b). The simplest way of measuring such a gas phase accumulation, is putting the culture into a syringe and record the gas production as the volume expansion reflected by the movement of the piston. But does it really work? If so, it is so trivial that somebody must have thought of it before. To our best knowledge and googling, we have been able to trace an origin back to studies of livestock digestion physiology, particularly the rumen fluid (Menke et al., 1979, Menke and Steingass, 1988, Duan et al., 2006). In those early days, only glass syringes were available and the field of application very restricted. We do not claim originality for the idea, but today, disposable syringes in suitable sizes are cheap and readily available, and the multitude of possible applications much wider.

There is a growing demand for reliable and low cost methods to investigate bio-reactions, especially in developing countries where research is poorly funded while there is a great demand for efficient environmental biotechnology. We have observed the usefulness of the presented protocol through research and education development aid projects. One goal of publishing this method is therefore to boost data generation regarding feed sources for biogas generation and pollution mitigation processes.

The main purpose of this current work is to present recommended procedures for widely different illustrative examples of syringe test applications, as well as some guidelines to avoid the major potential pitfalls of this approach.

2. Materials and methods

2.1. General aspects

Disposable plastic medical syringes (BD Plastipak, Franklin Lakes, NJ, USA) were applied with volumes 100 mL or 60 mL. After filling, syringes were closed air tight either by adding a needle and silicone rubber stopper, or directly by plastic closing cones (Braun, Melsungen, Germany). Stoppers with syringe valves (Mininert™ from VICI AG Int., Schenkon, Switzerland) were applied for sampling by suction to a connected syringe for later analysis of gas composition.

To test the variability in material properties of mass produced plastic syringes, a pressure friction test was run by filling 60 mL syringes with 40 mL of water, then carefully adding pressurized air until a volume expansion of 3 mL was achieved. The equilibrium pressure before and after expansion was recorded by an on line Beamex multifunction calibrator MC5 (Pietarsaari, Finland). This procedure was repeated in triplicate for each syringe examined.

Inocula were based on samples from natural sediments, from wastewater treatment plant, from biogas facilities at pilot or lab scale, as well as from long term adapted lab cultures. Types of inocula included suspended cultures, granules or moving bed biofilm carriers. See details below.

Incubations were performed at room temperature as well as in incubators (in house and Infors HT Minitron 22 C, Bottmingen, Switzerland) set at temperatures of 22, 25 or 35 °C. Stirring was obtained by a variety of laboratory shakers, including rotating (in Minitron at 110 rpm), linear (in house at 140 rpm) or tilting (Nutating Mixer, VWR, Radnor, PA USA), preferably with the syringes stacked in horizontal position.

A piston slide test was included in one of the initial experiments on denitrification (see below), where after 12 d of incubation, gas volumes denoted “before” were recorded directly, then the pistons were pulled and let slide back to a new stable position before re-reading the volumes denoted “after” for comparison.

2.2. Denitrification

Suspended inocula were obtained from a local river sediment (Nidelva, Trondheim) and from activated sludge of the wastewater treatment plant at Statoil's liquefied natural gas facility Hammerfest LNG, Melkøya. Biofilm cultures were obtained by long term lab enrichment cultures originating from local domestic wastewater, grown on moving bed biofilm reactor (MBBR) polyethylene carriers type Standard AnoxKaldnes K1 with 10 mm outer diameter (Veolia Water Technol. – AnoxKaldnes, Lund, Sweden).

The basal medium was prepared according to OECD guideline 301 on testing biodegradability (OECD, 1992) with nitrate added corresponding to 100 mg/L of NO_3^-–N. Syringes of 60 mL were filled with 40 mL of medium plus inoculum, emptied for air and closed before stacked in an incubator at 21 °C. Gas volumes formed were recorded daily unless otherwise stated. At end of incubation, nitrate concentrations left were determined by Hach–Lange assays for water quality (Hach-Lange, Düsseldorf, Germany) according to manufacturer's instructions.

Substrates tested included sodium acetate and a variety of amines applied in amine-based carbon capture and storage (CCS), such as monoethanolamine (MEA), diethanolamine (DEA), methyldiethanolamine (MDEA), 2-amino-2-methylpropanol (AMP) and piperazine (PZ). See also Henry et al. (2016a).

2.3. Biogas

The suspended anaerobic sludge inoculum was obtained from a long term UASB lab enrichment as described by Wang et al. (2013a, 2013b, 2013c), based on cultures originating from livestock manure, local river sediment, a domestic wastewater treatment plant sludge digester in Porsgrunn, Norway and granules from a UASB methane reactor treating wastewater from the pulp and paper industry at Norske Skog Saugbrugs AS, Halden, Norway.

In comparative tests of different mixed substrates, different organic mixtures resembling food wastes were made by mixing 1) apple juice as a source for carbohydrate, 2) yeast extract as a protein source and 3) cream as a source of fat, as shown in Table 1.

During amine based carbon capture and storage CCS, the solvent is recycled through a reclaimer for reuse, generating significant amounts of waste due to partial degradation. Such MEA-based reclaimer waste (MEAw) was collected at a MEA based CO_2 capture facility of a coal fired power plant. In general, such wastes do contain un-degraded amine as well as degradation products thereof, ammonia being the dominant one in addition to organic intermediates (Strazisar et al., 2003, Hauser et al., 2013). Test concentration was 20 g/L. A mixture of

Table 1
Composition of mixtures tested for biogas potential.

Sample name	Mixture of substrate	Volume ratio	COD ratio
A	Apple juice (carbohydrate) and yeast extract (protein)	45:55	~50:50
B	Apple juice (carbohydrate) and cream (fat)	95.5:4.5	~70:30
C	Yeast extract (protein) and cream (fat)	97.8:2.2	~80:20
D	Apple juice (carbohydrate), yeast extract (protein) and cream (fat)	44.6:53.2:2.2	~40:40:20