Biological production of methane and hydrogen from pig slurry and scotta

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Abstract
In recent years, the biological production of hydrogen (H_2) and methane (CH_4) in two-stage anaerobic systems has received increasing attention. In this work we evaluated in batch systems at laboratory scale the effect of decoupling the H_2 and CH_4 production phases on biogas yield. Scotta, a byproduct of ricotta cheese production, in co-digestion with pig slurry was used as substrate. Conventional anaerobic digestion (AD) at neutrality (*U*) was compared with dark fermentation followed by AD (*two-stage AD, D*). Four different inoculum sources were also compared, in U and in D. Methane amounts of 551 NmL g^{-1} volatile solids were measured on average in the D treatment at the end of AD. The process was faster in the reactors inoculated with methanogenic consortia. In the U treatment, only limited amounts of CH_4 could be produced, due to excessive acidification of the medium. Two-stage AD of pig slurry + scotta with selected methanogenic consortia has permitted faster production of higher CH_4 amounts than conventional AD.

Introduction
Several past and recent directives aim at containing the possible negative environmental impact of animal effluents in Europe. The exploitation of animal waste for energy production represents an important opportunity for the farmers to protect their incomes, eroded by the costs that farmers have to bear in the application of Directives. In the traditional anaerobic digestion (AD), hydrogen (H_2) production usually occurs simultaneously with methane (CH_4) production; H_2 however disappears as soon as it is formed, because it is quickly utilized by hydrogenotrophic methanogens. In recent years, the separated biological production of H_2 and CH_4 in two-stage anaerobic systems has received increasing attention [1]. Decoupling these processes should permit, together with the production of H_2, also an increase in CH_4 production. In this work we evaluated the effect of decoupling the H_2 and CH_4 production phases on overall biogas yield using pig slurry in co-digestion with scotta as AD substrate. Scotta is deproteinized whey resulting from the production of "ricotta", a typical Mediterranean cheese. Cheese whey, having a composition similar to scotta, is a well known source of biohydrogen [2], whereas it is reported to inhibit CH_4 production when used in codigestion with pig slurry, in the absence of pH control [3].

Material and Methods

Experimental design
Compared treatments were: I) AD at neutrality (conventional AD, *U*), and II) dark fermentation followed by AD (two-stage AD, *D*); 4 different inoculum sources were also compared, in U and in D, in a completely randomized experimental design with 3 replications.

Substrates
A sterilized mixture of fresh pig slurry and scotta in a 70:30 ratio was used as substrate, both in U and in the I stage of D. The scotta percentage contribution to the mixture was chosen deliberately much higher than that indicated as prudential for traditional AD (cheese whey contribution to the codigestion mixture as suggested by practitioners: <10%; [4]). Pig slurry was collected from the effluent storage tank of our experimental farm, before the solid-liquid separation step. It was boiled and filtered before use. This treatment was applied because, even though it modifies to some extent the original pig slurry composition, it makes easier the following steps of substrate preparation. Scotta (pH 6.0, lactose content 5.6%) was obtained from a dairy farm near our Research unit. The mixture pig slurry + scotta had the following composition: total solids (TS), 2.63%; volatile solids (VS), 87% TS; total Kjeldahl N (TKN), 0.54 g L^{-1}; NH_4-N, 75% TKN; pH 7.2. The fermented broth was used for the II stage of D, 7

days after the start of the incubation. Its pH was adjusted at the value of 7.20 by means of Na bicarbonate and NaOH, just before inoculum of the II stage. A suitable amount of the same reaction mixture used for the production of the fermented broth (inoculum included) was stored at -20 °C, and utilized as codigestion mixture in the reactors of the U treatment.

Inocula

Anaerobic digestion, both in the U treatment, and in the second stage of the D treatment, was carried out using 2 selected methanogenic consortia (BF and MA08), separately or in combination. Fresh pig slurry, that is the liquid fraction coming from solid-liquid separation of pig manure, was used as control inoculum ("S3"), in U and in the stage I of D. This inoculum was chosen with the goal to equally promote all the microbial species initially present in fresh pig slurry; in fact, anaerobic digestion of pig slurries normally relies on these wild populations. The BF consortium was obtained by starvation of S3, and included wild anaerobic microbial species, whereas MA08 was obtained by means of a standard methanogen-selection procedure. The prevailing microbial group in this consortium is represented by acetoclastic methanogens, as verified by means of standard cultural techniques. In U, these consortia were compared with S3. In D, they were inoculated on fermented broth and compared with non-reinoculated fermented broth (NRI). In both treatments the inoculum was collected, centrifuged and washed in strictly anaerobic conditions to increase its activity and to improve the uniformity of distribution in the various reactors. These inocula did not show any endogenous hydrogenogenic or methanogenic activity.

Batch laboratory incubations

The hydrogenogenic phase (first stage of the simulated 2-stage process), in D, was carried out in 500-mL reactors (reaction mixture: 200 mL substrate + 200 ml inoculum), at 35 °C, in anaerobic conditions (N_2 as filling gas in the head space [HS] of the reactors). Anaerobic digestion was performed simultaneously, for U and D. Incubations were performed in 100-mL reactors containing 50 mL of substrate, in the dark, in strictly anaerobic conditions (CO_2/N_2 5/95, in the HS), at 35 °C.

Chemical analyses

Biogas volume and composition during the I stage of D was monitored over a week (after 2, 5 and 7 days from the start of the incubation. Biogas volume and composition in U and in the II stage of D were measured during a 70-d period. In the large majority of the reactors the AD had stopped after 7 weeks (49 d), and here we report data relevant to this period. Biogas volume was measured by means of glass syringes, according to Owen *et al*. [5]. Gas composition was determined on the samples collected for the measurement of gas volumes, by means of a MicroGG Agilent 3000.

Results and discussion

Hydrogen production in the I stage of the D treatment (dark fermentation)

The maximum H_2 production was measured 2 days after the start of the incubation (42.3 _mL g^{-1} VS, on average). It was nearly completed after a week. This fermentation reduced the VS content of the reaction mixture from the initial value of 2.30% to 1.11%, and it caused a strong dropping of the initial pH value (from 7.20 to 4.5, on average), in a few days.

Biogas production in U

A week after the start of the incubation, only traces of CH_4 (1.14 mL, on average) were produced in the reactors of the U treatment (Table 1). Hydrogen was 28.2% of the overall biogas production in U (22.1 mL, on average, corresponding to 19.0 mL g^{-1} VS). After this period, H_2 disappeared quickly from the HS of the reactors. Seven weeks after the start of the incubation, biogas production (89.8 mL, on average) had increased by only 11.3 mL (14.4%) in comparison with that measured after a week (78.5 mL). No H_2 was detected in the HS. Only 2.4 mL of CH_4 were obtained, on average. The low CH_4 production in the single-stage process was attributed to the acidifying effect of scotta. A negative effect of high concentrations of cheese whey on methanogenesis has been reported [2].

Biogas production in D

A week after the start of the incubation, biogas in the reactors of the D treatment (II stage) contained large amounts of CH_4 (69.8 mL, on average; from Tab. 1), whereas H_2 was absent. Seven weeks after the start of the incubation, the cumulated biogas production in D was on average 460 mL (+ 354 mL, in comparison with the mean production after a week, 106 mL), 338 ml (73.5%) being constituted by CH_4 (526 NmL CH_4 g^{-1} SV).

Influence of microbial consortia

A week after the start of the incubation, in the U treatment no significant differences on H_2 production were detected between inocula, in comparison with the control S3. This is due to the fact that inocula had been not selected for H_2, but for CH_4 production. Hydrogen production could be attributed to the prevailing of acidogenic microorganisms deriving from the initial inoculum, able to overcome the other groups due to a selective pressure exerted by the substrate and, at the same time, to tolerate a strong and fast decrease of the pH values. The amount of H_2 produced in the U stage was however much lower (45%, on average) than that produced in the hydrogenic stage (I stage) of the D treatment using the S3 inoculum.

In the II stage of the D treatment significant differences of biogas and CH_4 production were detected among microbial consortia. The acetoclastic methanogenic consortium MA08 produced the highest amount of CH_4, followed by BFMA08, BF and NRI. This is in agreement with the fact that the MA08 consortium, unlike BF, was specifically selected for the utilization of substrates rich in acetate, like those deriving from the hydrogenogenic fermentation of carbohydrate-containing substrates.

Methanogenesis was accelerated by the use of selected consortia: a wk after the start of the incubation, the D reactors inoculated with MA08, alone or in combination with BF, had an advantage of + 98 mL of CH4, in comparison with S3, producing 6 mL CH_4, on average; and BF alone had produced 60 mL more than S3.

Seven weeks after the start of incubation the average CH_4 production in the D stage was 338 mL, corresponding to 551 NmL g^{-1} VS, without differences among microbial consortia in the final amount of CH_4 produced. This was somehow expected, because the amount of CH_4 produced by a given substrate depends on the amount of available easily-decomposable fraction of VS in the substrate, at least below the occurring of overload or inhibition thresholds [6].

These results clearly indicate that the use of selected consortia may positively shorten the time for AD.

Table 1. Biogas production (cumulated values) 7 and 49 d after the start of incubation, and final values of pH and VS concentration in a mixture of pig slurry and scotta inoculated with different microbial consortia. U: conventional AD; D: two-stage AD. NRI: non reinoculated.

		7 d			49 d			
Treatment	Inoculum	Biogas, mL	H_2, mL	CH_4, mL	Biogas, mL	CH_4, mL	pH	% VS, residual
U	S3	75	19	2	86	6	3.6	1.47
U	BF	79	23	1	91	1	3.6	1.29
U	MA08	83	25	1	93	2	3.6	1.28
U	BF+MA08	78	22	1	90	1	3.6	1.25
D	NRI	31	0	6	471	340	7.7	0.67
D	BF	106	0	66	459	336	7.6	0.62
D	MA08	148	0	109	446	335	7.5	0.62
D	BF+MA08	138	0	98	462	340	7.6	0.57
LSD (α=0.05)		*7.9*	*4.4*	*2.5*	*14.6*	*15.1*	*0.03*	*0.04*

Changes in substrate composition

Large differences were observed in the composition of the substrate, at the end of the AD. In particular, the pH of the U treatment had dropped from 7.2, at the start of the incubation, to very low pH values (3.6, on average, without differences among inocula), analogously to what had happened in

the I stage of the D treatment, whereas in D the pH had increased to 7.6. Meanwhile, the residual VS in D was 0.62% on average, much lower than in U (1.32%). These changes imply a reduced digestion of the substrate, in U, together with the occurrence of anomalous fermentations, as also shown by the high mean CO_2 values detected in the HS of this treatment (42% of CO_2 in U vs 29%, in D, after 49 d; data not shown).

Conclusion and perspectives

The decoupling of the hydrogenogenic and methanogenic steps in biogas production obviates to the risk of substrate acidification, when using scotta in codigestion with pig slurry, and allows high methane yields to be obtained. The disposal of scotta is costly, due to its high organic load; biogas production from scotta in codigestion with pig-slurry, in 2-stage systems, reduces its polluting load. The use of selected methanogenic consortia improves methane production.

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