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Methane Production from Solid Potatoes by a Procedure Simulating a Bench-Scale Sequencing Batch Reactor Anaerobic Process

I. Colussi, A. Cortesi,* V. Gallo, A. S. Rubesa Fernandez, and R. Vitanza
Department of Engineering and Architecture,
Piazzale Europa, I-34127, Trieste, Italy

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In this study, an experimental setup for the evaluation of a two-stage anaerobic digestion has been developed: a laboratory-scale apparatus was assembled employing solid potatoes as energy crops. Two coupled 5-litre batch-fed stirred reactors, one for the hydrolytic-acidogenic step and one for the acetogenic-methanogenic step, kept at mesophilic temperature (308.1 K), were adopted. Evaluated in the first acidogenic reactor was the influence of fermentative yeast (*Saccharomyces cerevisiae*) on the degree of hydrolysation and on the acidification rate of the vegetable substrate. All runs were performed without the addition of chemicals. Samples of hydrolysed substrate were sent to the second methanogenic bioreactor, fed with an industrial anaerobic sludge and selected lyophilized anaerobic bacteria, in order to evaluate the methane yield of the produced biogas and the specific methanogenic activity (SMA). The whole procedure was repeated simulating an anaerobic sequencing batch reactor (ASBR) process.

Key words:
Bioreactors, anaerobic process, biogas, potatoes, yeast, ASBR

Introduction

It is well known that anaerobic digestion (AD) is a multi-step process in which complex substrate molecules are firstly hydrolysed to simpler soluble monomers, in the hydrolytic-acidogenic step, then transformed into volatile fatty acids (VFAs), and finally converted into methane and carbon dioxide, in the acetogenic-methanogenic step.[1–3]

Normally, bacteria involved in these steps are quite different and therefore present maximum growth rate under different conditions. For example, hydrolysis is reported to be the rate-limiting step in the whole AD process with complex substrates, requiring often slightly acid pH.[4] On the other hand, methanogenesis has its optimum for slightly alkaline pH. Two-stage AD appears to be very suitable for substrates with high insoluble COD (Chemical Oxygen Demand) contents like fruit and vegetable wastes, owing to the possibility of carrying out the stages in different environments. This operative mode increases stability, efficiency and conversion rate.[5–9] The hydrolysis can be improved by inoculating the reactor with microorganisms producing hydrolytic enzymes specific for the substrate.[7] However, in spite of these advantages, two-stage AD processes are not commonly applied in industrial application,[10] probably because there is the need for more exhaustive studies to motivate higher plant investment. Recent interesting reviews on conventional, two-phase (high-rate) and temperature-phased anaerobic digestion processes are reported by Demirel *et al.*[11] and Lv *et al.*[12].

The aim of this work is the proposal of a procedure to simulate an anaerobic sequencing bench-scale reactor (ASBR). Solid potatoes, i.e. complex substrates, are employed as energy crops. A first stage process conducted by *Saccharomyces cerevisiae* (SC) yeasts was compared with a process led by the heterotrophic biomass grown on the same substrate, then both hydrolysed systems were sent to the acetogenic-methanogenic reactors, inoculated with an industrial anaerobic sludge and selected lyophilized anaerobic bacteria, to point out potentiality in biogas conversion and methane yield.[13,14]

Materials and methods

Experimental setup

Experiments were performed in two identical double-stage apparatuses, each consisting of two coupled mesophilic glass 5 L reactors: reactors 1 and 2 for the hydrolytic-acidogenic stage, and reactors 3 and 4 for the acetogenic-methanogenic step. The bioreactors were coupled, as reported in Fig. 1a, to simulate an anaerobic sequencing batch reactor (ASBR).

Bioreactors were contained in a temperature-controlled water bath (308.1 ± 0.1 K) and mixed

*Corresponding author: Prof. Angelo Cortesi;
E-mail: angelo.cortesi@di3.units.it; Tel: 39 040 558 3755;
Fax: 39 040 569823

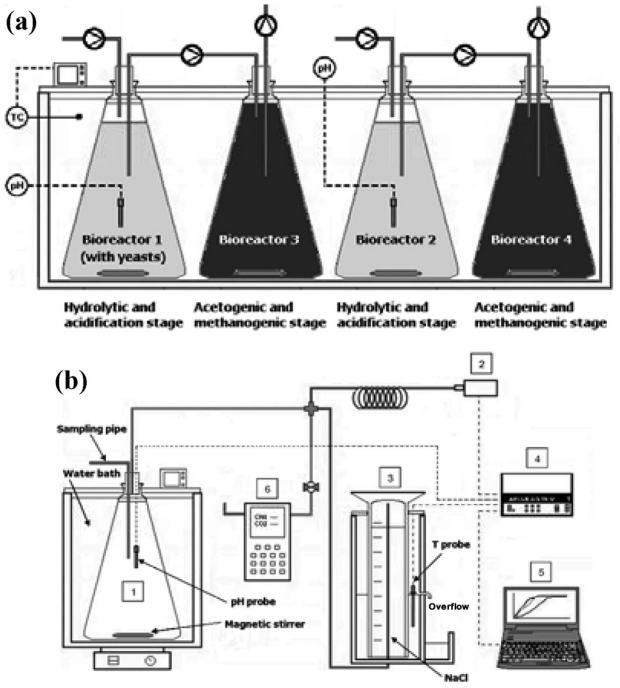

Fig. 1 – *(a) Experimental setup: simulation system of an ASBR digester. (b) Schematic diagram of the experimental apparatus: 1 Bioreactor; 2 P transducer: 3 Gas meter; 4 Data logger; 5 Computer; 6 Gas analyser.*

continuously with magnetic stirrers (ARE, Velp Scientific). The system temperature was controlled using a heating immersion circulator (Julabo MB).

A schematic diagram of a single batch unit is shown in Fig. 1b. Each reactor was connected by a stainless-steel pipeline to a height type gasometer, a 2 L volume glass cylinder turned upside-down, with an internal cross-sectional area of about 50 cm^2 and a fixed height from the external liquid surface maintained by an overflow system.

The gasometer was put inside a plexiglas vessel filled with a saturated solution of NaCl to lower CO_2 solubility.[15] Biogas pressure and barrier solution temperature were monitored by a differential pressure transducer (RS Instruments) and a platinum resistance thermometer (PT100 probe); compositions of CH_4, CO_2, N_2, O_2, H_2, H_2S were acquired by a gas analyser (GA 2000 plus, Geotechnical Instruments). pH probes (Hanna HI 62910) were introduced in the acidogenic bioreactors to outline the pH changes during the test. All the data were continuously recorded by a data logger (Agilent) controlled by a PC. The starting setup was obtained by putting the system under vacuum conditions (see Fig. 2).

Measurement of the produced biogas

According to Walker *et al.*,[15] biogas volume to standard conditions (T_{stp} = 273.15 K, p_{stp} = 100 kPa) V_{stp} [L] is calculated by equations 1 and 2, with the

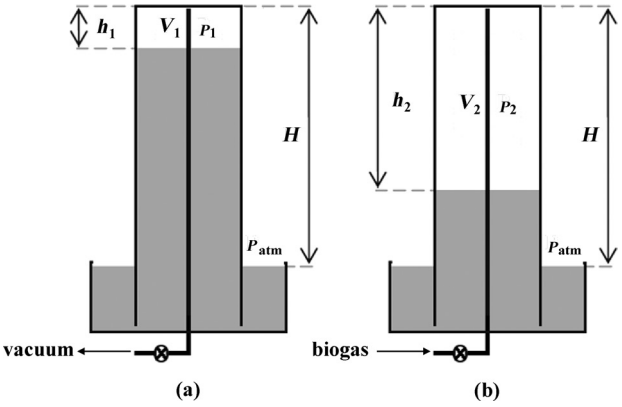

Fig. 2 – *Gasometer scheme for biogas volume calculation. a) Start setup, b) Final condition.*

following assumptions: the biogas acts as a perfect gas, the height gasometer cross-sectional area A is constant, once leaving the anaerobic digester, the biogas quickly cools to ambient temperature, the biogas is saturated with vapour and the saturated vapour pressure can be modelled by the Goff-Gratch equation.

$$V_{stp} = \frac{T_{stp} \cdot A}{T_{atm} \cdot p_{stp} \cdot 10^3}\left[\left(p_{atm} - p_{T_{atm}}^{H_2O} - \Delta p_2\right) \cdot h_2 - \left(p_{atm} - p_{T_{atm}}^{H_2O} - \Delta p_1\right) \cdot h_1\right] \quad (1)$$

$$\Delta p_i = \rho_b \cdot g \cdot \left(H - h_i\right) \quad (2)$$

where:

$p_{T_{atm}}^{H_2O}$ = saturated vapour pressure at atmospheric temperature, [Pa];
p_{atm} = atmospheric pressure, [Pa];
T_{atm} = atmospheric temperature, [K];
A = constant cross-sectional area of the gasometer, [m^2];
h_i = gap between gasometer liquid surface and external liquid surface, [m];
Δp_i = differential pressure monitored by pressure transducer, [Pa];
ρ_b = density of the barrier (saturated NaCl) solution, = 1.198 [kg m^{-3}];
H = constant gap between the top of the gasometer and the external liquid, [m].

Specific methane yield and specific methanogenic activity SMA

The specific methane yield is an index used to describe the efficiency in the substrate-to-biogas conversion: therefore, it provides a quantitative evaluation of the methane production. It can be defined as the ratio between the total methane produced and the converted substrate, often expressed in grams of total solids TS (considered as dry fraction), grams of total volatile solids TVS or grams of COD.

The specific methanogenic activity (SMA) test indicates the efficiency of the anaerobic treatment because it measures the rate of the methanogenic phase and, considering the quantification of the active biomass, it also evaluates the reactor methanogenic capacity.[16] It is an effective index for determining the maximum applicable organic loading rate (OLR), and it is based on the maximum methane productivity rates, expressed as (mL CH_4 g^{-1} VSS h^{-1}) or (g COD g^{-1} VSS h^{-1}). Aquino *et al.*[17] suggest the conditions required to perform a reliable SMA test: it has to be conducted on acclimatized sludge with concentration between 2.5 and 20 gVSS l^{-1}, specifying a food-to-microorganism ratio (F/M) for every concentration.

Substrate preparation

The substrates used in this study were Agata variety potatoes, collected from an agro-industrial company of the Friuli Venezia Giulia region (Italy).

Solid potatoes were not washed or sterilised before the acidification stage, in order to simulate an industrial application and to allow natural bacterial contamination.

Potatoes were shredded into small particles (< 3 mm), homogenized using a kitchen blender and analysed: the substrate characteristics in terms of total solids TS, volatile total solids TVS, moisture and total COD are reported in Table 1.

Table 1 – *Characteristics of the potato substrate*

TS [gTS g^{-1}]	TVS [gTVS g^{-1}TS]	Moisture [%]	tCOD [mg g^{-1}]
0.156	0.940	84.0	180.1

The prepared potatoes were then mixed with distilled water in a ratio of 1 : 8 by weight to allow magnetic mixing and to promote substrate diffusion and bacterial growth. These samples were formerly adopted for a pre-study of the hydrolytic and acidification stage.

Study of the hydrolytic and acidification stage

The hydrolytic stage was investigated, in a pre-study test, by means of two reactors loaded simultaneously: the first (bioreactor 1 of Fig. 1a) with the addition of 0.1 g L^{-1} of Saccaromyces Cerevisiae (SC) yeast (Fermol® Arome Plus), purchased from AEB SpA, and the second (bioreactor 2 of Fig. 1a) without additional active biomass. All the experimental conditions are reported in Table 2. pH variations and CO_2 productions were then measured to obtain the influence of SC on the degree of hydrolysation and volatile fatty acids (VFA) production. From the pre-study, the hydraulic retention time (HRT) of the acidogenic reactors was evaluated: after this time, samples from acidogenic reactors were sent to the methanogenic reactors by peristaltic pump.

Table 2 – *Starting conditions for hydrolytic and acidification pre-study*

	Bioreactor 1	Bioreactor 2
Potato Mass [g]	625	625
Total COD [g]	110.0	100.2
Distilled Water [L]	4.350	4.350
Added active biomass [mg L^{-1}]	97(*)	–

(*)*Saccharomyces cerevisiae* (Fermol® Arome Plus)

Acetogenic and methanogenic stage

The acetogenic-methanogenic reactors were initially inoculated with anaerobic sludge from full scale Up-flow Anaerobic Sludge Blanket (UASB) operating in Friuli Venezia Giulia treating brewery wastewater and with selected lyophilized anaerobic bacteria (Bioactiva B 37.00, 20 g m^{-3}).[18]

Bioreactors 3 and 4 (Fig. 1a) operated 60 days with synthetic substrate feedings, ethanol and sodium acetate, selected to acclimatize the methanogenic bacteria. In Table 3, the characteristics of the two systems at the end of the acclimatization period are reported.

Table 3 – *Characteristics of the methanogenic starting systems*

	TSS [g L^{-1}]	VSS [g L^{-1}]	tCOD [g L^{-1}]	sCOD [g L^{-1}]
Bioreactor 3	11.40	3.68	11.60	0.176
Bioreactor 4	13.72	3.48	13.80	0.172

ASBR simulation

A cyclic process was adopted to simulate an ASBR digester: samples of hydrolyzed substrates were taken out of bioreactors 1 and 2, and led to the methanogenic reactors (3 and 4), whereas new potato substrates, mixed with water, were placed for the hydrolytic and acidification stage.

Because of the constant value of the hydraulic retention time (HRT), the organic loading rate (OLR) was a direct function of the COD transformed into methane.

Analytical methods

Total solids (TS), total volatile solids (TVS), total suspended solids (TSS) and volatile suspended solids (VSS) were determined from slurry sample of 25 mL collected from the bioreactors following Standard Methods.[19]

Soluble COD values were measured by spectrophotometric technique (Hach DR/2010) from filtered samples of 25 mL through 0.45 μ filter paper at the beginning and end of the experiment.

pH measurements were performed with a pH meter (Hanna Instruments – HI 98150). Total COD data of slurries, substrates and digested residues were measured by a modified and optimized dichromate reflux method for solid substrates and solutions with high suspended solid content.[20] Biogas composition (CH_4, CO_2, O_2) was analysed by infra-red absorption gas analyser (GA 2000 plus, Geotechnical Instruments).

Results and discussion

Hydrolytic and acidification stage

The results obtained in the pre-study test are summarized in Fig. 3: the acidogenesis process is verified since the starting of both bioreactors, but the addition of *Saccharomyces cerevisiae* (bioreactor 1) produces a greater amount of biogas with a two step performance in the diagram. The presence of a second step in biogas production (after 25 h) is given by the yeasts contribution to the process. Besides, fermentative SC and potato microorganisms have a synergic action in bioreactor 1 obtaining the first step in a shorter time (about 8 h instead of 15 h).

The composition of the produced gas, after 40 h of experiment, is shown in Table 4: bioreactor 1 produces a greater amount of CO_2, due to the capability of yeasts to work in strong acid conditions.

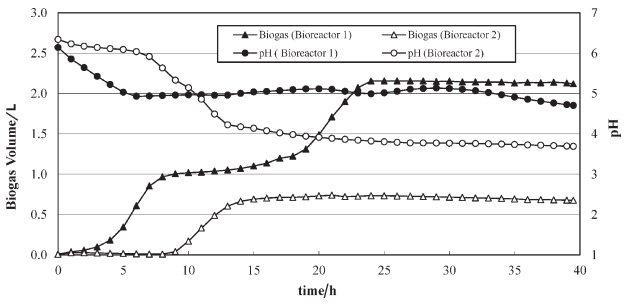

Fig. 3 – *Biogas production and pH values for potato substrates hydrolysis and acidification stage*

Table 4 – *Biogas production in hydrolytic and acidification stage*

	Bioreactor 1	Bioreactor 2
Biogas production (*) [L]	2.120	0.700
CH_4 [%]	0.1	0.1
CO_2 [%]	67.0	16.5
O_2 [%]	4.1	6.5
H_2 [ppm]	> 1100	> 1100
H_2S [ppm]	50	265
Other (**) [%]	28.8	76.9
Total CO_2 subtracted [L]	1.420	0.115
Final pH	4.7	3.7

(*) Volume (V_{stp}) of biogas produced with HRT of 40 h
(**) The balance to 100 % of the analyser (mainly H_2, and N_2 from loading)

Actually potato microorganisms are inhibited at pH value of 4.5 and pH drops slowly around 4.5 with SC and 3.5 without fermentative yeasts. It is important to note that for about 15 h the amount of the produced biogas is comparable in both bioreactors: after this time, the action of the yeasts trebles the production. From these results, 15 h were considered as the starting hydraulic retention time (HRT) in acidogenic reactors 1 and 2. Moreover, it is interesting to consider the high value of the balance to 100 % of the analyser in bioreactor 2: this is probably related to a greater production of H_2 (not measured over 1100 ppm by the analyser) in respect to bioreactor 1.

ASBR simulation: hydrolytic and acidification stage

The influence of *Saccharomyces cerevisiae* on the hydrolytic and acidogenic step was also tested in the laboratory-scale ASBR digester (Fig. 1a).

The hydrolytic-acidogenic step was repeated with the same conditions (Starting load) of the pre-study test, with and without addition of yeasts: the first transfer (RUN 1) was done after the hydraulic retention time of 15 h. Then, samples collected from the acidogenic reactors (0.250 L) were sent to the methanogenic reactors by peristaltic pumps, replacing equivalently removed volumes of sludge.

Repeating the same procedure, a second transfer (0.260 L) from hydrolytic-acidogenic reactors (RUN 2) was carried out 7 days later, the time required to reach complete endogenous conditions in methanogenic reactors. At the same time, new potato substrates (150 g), shredded but not mixed with water, were replaced in both hydrolytic bioreactors to occupy the empty volumes.

Table 5 – *Loadings, total and soluble COD values for bioreactors 1 and 2 in ASBR simulation*

	COD loaded [g]		COD transferred [g]		Exp tCOD [g L^{-1}]		Exp sCOD [g L^{-1}]	
Bioreactor	1	2	1	2	1	2	1	2
Starting load (HRT 15 h)	110.0	100.2			22.00	20.04	5.84	5.48
RUN1 (HRT 7 days, day 2)			5.50	5.01	20.90*	19.04*		
End of RUN1 (day 9)					17.80	18.70	15.73	7.23
RUN2 (HRT 7 days, day 9)	27.00	27.00	4.63	4.86	22.27*	23.13*		
End of RUN2 (day 16)					20.70	21.75	19.70	8.04
RUN3 (HRT 7 days, day 16)	25.00	25.00	4.35	4.57	24.83*	25.84*		
End of RUN3 (day 23)							19.86	9.16

(*) Calculated values

A cyclic process was then adopted to simulate an ASBR digester: hydraulic retention time (HRT) was kept at value of 7 days, and the biogas produced, methane yield, and SMA were calculated as described previously. Therefore, after 7 days of the methanogenic stage, new samples of hydrolysed substrates (0.210 L) were taken out and led to the methanogenic bioreactors (RUN 3), and potato substrates (140 g) were fed again in both bioreactors 1 and 2.

In Table 5, all COD transfers in hydrolytic and acidogenic bioreactors 1 and 2 are summarized with total and soluble COD values measured at the end of each RUN (i). It is important to note that the last soluble COD value for the system with SC yeasts (19.86 g L^{-1}), was about 3.5 times greater than the starting value (5.84 g L^{-1}) and twice that of the final soluble COD value of the system without fermentative yeasts (9.16 g L^{-1}). This result is validated also by the feature of the two systems: homogeneous (for SC contribution) in bioreactor 1 and heterogeneous (still presence of solid substrate with difficult measurement of tCOD) in bioreactor 2.

For both hydrolytic bioreactors, average pH values were established at 3.7, but a significant biogas production was evidenced only in bioreactor 1.

ASBR simulation: acetogenic and methanogenic stage

Biogas productions and compositions, obtained in methanogenic reactors, are reported in Table 6, in which methane yield is related to the amount of the loaded COD (COD_{in}). According to the mass balance, the methane gas at an average gasometer temperature of 273.15 K, approaches the maximum value of 0.38 m^3 per kg of COD removal (0.35 m^3 at STP conditions[21]): from Table 6, this theoretical methane volume is approximated in bioreactor 3 after 168 h of RUN 3.

Table 6 – *Biogas production in methanogenic reactors*

Bioreactor	RUN 1		RUN 2		RUN 3	
	3	4	3	4	3	4
Transferred Volume [L]	0.250	0.250	0.260	0.260	0.210	0.210
Loaded COD_{in} [g]	5.50	5.01	4.63	4.86	4.35	4.57
Biogas production(*) [L]	1.640	1.255	3.610	3.160	2.635	2.035
CH_4 [%]	65.6	61.4	59.8	46.2	59.2	56.3
CO_2 [%]	23.0	10.2	37.5	29.1	37.8	35.4
O_2 [%]	3.2	6.2	1.0	4.4	0.8	1.7
H_2 [ppm]	> 1100	> 1100	170	310	126	188
H_2S [ppm]	2	5	178	63	31	22
Other(**) [%]	8.2	22.2	1.7	20.3	2.2	6.6
Produced CH_4 [L]	1.075	0.770	2.160	1.460	1.560	1.145
Methane yield [L CH_4 g^{-1} COD_{in}]	0.20	0.15	0.47	0.30	0.36	0.25
Efficiency(***) [%]	52.6	39.5	123.7	78.9	94.7	65.8

(*)Volume (Vstp) of biogas produced with HRT of 7 days (168 h)
(**)The balance to 100 % of the analyser
(***)[Methane yield/Theoretical Methane yield (= 0.38 L g^{-1}COD at 100 kPa and 273.15 K)] ·100

As expected from the soluble COD values of the feeds, after three runs (three weeks of experiments), bioreactor 3 evidences an optimal efficiency (ratio between produced and maximum theoretical CH_4 volumes) of 94.7 % compared to the 65.8 % of bioreactor 4. The produced biogas amount in the bioreactors is about 2.6 l for bioreactor 3, and about 2 l in bioreactor 4: CH_4 percentage in bioreactor 3 is greater (59.2 %) than in bioreactor 4 (56.3 %).

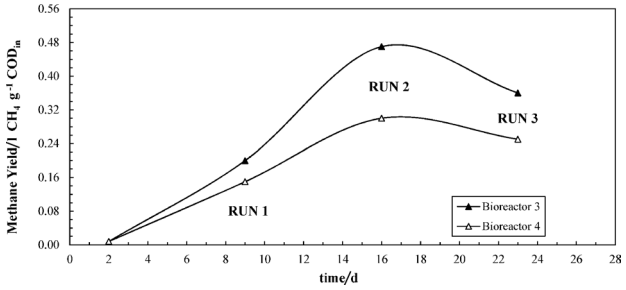

Fig. 4 – *Yields in methanogenic bioreactors (dashed line refers to theoretical methane yield of 0.38 L $g^{-1}COD_{in}$). The curves are drawn to show trend in data.*

The influence of the hydrolysis step with yeasts on the gas production is displayed in Fig. 4: the sinusoidal trend of the methane yield, due to the not yet reached steady state regime, in reactor 3 is much closer to the optimum value (0.38 L g^{-1}COD) compared to the methane yield of the reactor 4, that does not exceed the value of 0.30 L g^{-1}COD.

Fig. 5 presents the comparison of specific methane yield values obtained in RUN 3 for bioreactors 3 and 4.

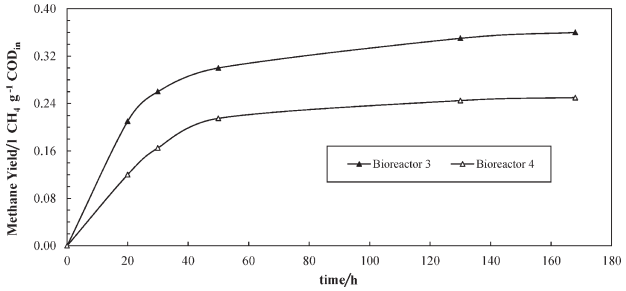

Fig. 5 – *Experimental methane yields in RUN 3 for methanogenic bioreactors (dashed line refers to theoretical methane yield of 0.38 L $g^{-1}COD_{in}$). The curves are drawn to show trend in data.*

To verify the reliability of the experiments, calculated were the raw media values of the total efficiency of the methanogenic systems, expressed as ratios between produced and theoretical CH_4 volumes in the three RUNS: an indicative result of 87.1 % is obtained by bioreactor 3, whereas 61.5 % is the efficiency exhibited by bioreactor 4. Thus, the average volume percentages of CH_4 in the three RUNS in bioreactors 3 and 4 were 60.8 % and 52.3 %, respectively.

The higher amount of CO_2 in bioreactor 3 appears interesting and unexpected: this fact probably correlates with the significant presence of oxygen (air) in bioreactor 4 still after three weeks of experiments with an O_2 percentage going down from 6.2 % in RUN 1 to 1.7 % in RUN 3 (bioreactor 3 from 3.2 % to 0.8 %).

Experimental SMA trends in methanogenic bioreactors, for RUN 3, are reported in Fig. 6: for both reactors displayed is the complexity of the hydrolysed substrates, whereas the velocity of the methanogenic phase becomes very low after 40 h of experiment (close to endogenous conditions).

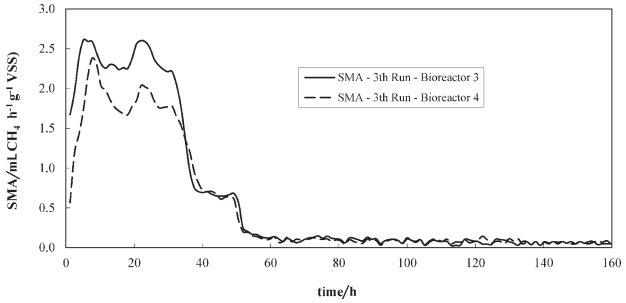

Fig. 6 – *Experimental SMA values in RUN 3 for methanogenic bioreactors.*

The maximum SMA value (over 2.5 mL CH_4 h^{-1} g^{-1}VSS) is reached after 6 h in reactor 3, fed with substrate from bioreactor 1, while a lower value is stated in reactor 4: these results are clearly related to *Saccharomyces cerevisiae* contribution in the first hydrolytic and acidification step.

Another important feature for both methanogenic bioreactors are the stable VSS values measured during the experiments, with final VSS levels of 3.48 g L^{-1} and 3.24 g L^{-1} for bioreactor 3 and 4, respectively, as compared to the initial values reported in Table 4 (3.68 g L^{-1} and 3.48 g L^{-1}). This means a constant occurrence of anaerobic biomass in bioreactors, that it is very important for the scale-up of industrial continuous stirred-tank reactor (CSTR) processes.

Conclusions

In this work, a laboratory-scale apparatus for the evaluation of a two-stage anaerobic digestion has been presented, employing potatoes as energy crops and introducing a procedure to simulate an ASBR process. In the first hydrolytic-acidogenic step, the influence of *Saccharomyces cerevisiae* was verified and the system with yeasts trebled the biogas production, with a final soluble COD twice that of the soluble COD for the system without yeasts.

Samples of pre-hydrolysed substrate were sent with a cyclic process to the acetogenic-methanogenic phase to simulate an ASBR system (HRT = 7 days): also in this case, the influence of the used *Saccharomyces cerevisiae* was recognized, obtaining higher values in efficiency and biogas production, and confidence in a close steady state regime after 23 days of experiment.

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