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METHANOGENIC AND ACIDOGENIC ACTIVITY TEST IN AN ANAEROBIC THERMOPHILIC REACTOR

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SUMMARY

The experimental conditions selected (addition to assay medium of micronutrients, macronutrients and pH control), the experimental design and the procedure for maximum acidogenic and methanogenic activity determination tests in thermophilic biomass, are presented. The proposed tests are highly reproducible and can be carried out in a short space of time. Specific tests are applied to measure the maximum acidogenic and methanogenic activities in lab-scale anaerobic reactors treating wine-distillery wastes in the thermophilic range (55°C).

INTRODUCTION

The alcohol for beverage industries in Spain is produced from distillation of various feedstocks, such as fermented sugar, beet molasses and wines. The residual liquid waste after distillation of the alcohol is called vinasse, which is highly with organic compounds polluted and can have a widespread detrimental effect on the environment if it is discharged untreated. Anaerobic digestion is a well established technology for treatment of vinasses. Also, as the vinasses are discharged at high temperature, thermophilic anaerobic digestion is of interest for the treatment of this waste and, indeed, thermophilic processes have been found to be higher than mesophilic processes in terms of removal rate of organic matter. However a drawback of thermophilic anaerobic digestion consists in that when the process is subjected to a sudden change in operating parameters (which is habitual in the treatment of vinasses due the variations in the waste flow rate and organic concentration) digestion becomes unbalanced due to the fact that the various metabolic groups of bacteria respond in a different manner. For this reason, knowledge of specific activities of the two principal bacterial groups involved in anaerobic digestion, namely acidogenic and methanogenic floras, could be very useful as a process control indicator in thermophilic anaerobic digestion.

A number of authors has attempted to characterize anaerobic reactor biomass, some in terms of microbial ecology, others in terms of biological activity (measured as utilization rate of different substrates or end products generated). Numerous researchers have proposed batch bioassays to determine the activities of anaerobic cultures in anaerobic ecosystems (Oven et al., 1979; Valcke and Verstraete, 1983; Dolfing and Bloemen, 1985; James et al., 1990). The specific

activity test also allows the prediction of maximal space loading rate, which can be applied during start-up procedures, and could be very useful for the selection of an adapted sludge for inoculation processes (Soto et al., 1993), for the detection of an unbalanced situation between several cultures in a reactor, for the calculation of the maximum organic load rate in a reactor (Field et al., 1988), etc.

However, all these papers report research are referred principally to biological activity tests in the mesophilic range of temperature, and no information is available for measuring acidogenic and methanogenic activity in the thermophilic range. The aim of this paper is to select the best conditions (methodology and procedures) for carrying out batch bioassays tests to determine acidogenic (AA) and methanogenic activity (MA) in thermophilic anaerobic reactors treating vinasses.

EXPERIMENTAL METHODS

Materials: The activity test procedure involves incubating the sludge from a thermophilic lab-scale digester with a specific substrate according to the microbological group being tested in sealed anaerobic vials. Vials of 125 ml with rubber stoppers and crimp seals are used. For specific methanogenic activity tests, the experimental equipment includes a device to measure the methane generated. This device consists of an inverted flask of 250 ml containing an alkaline solution (0.6 M NaOH or KOH) that allows the selective measurement of methane (see Figure. 1).

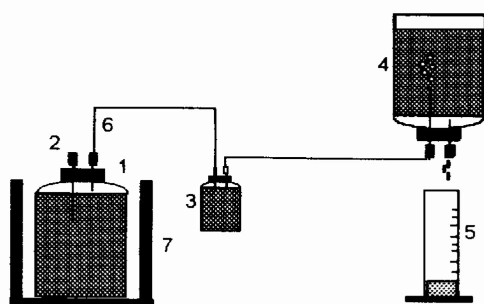

Figure-1. **Experimental equipment used to determine methanogenic activity: 1. Assay reactor. 2. Sampling point. 3. Security vessel. 4. Gas-meter. 5. Calibrated cylinder. 6. Biogas bleed-off. 7. Thermostatic bath.**

Methodology. A series of batch experiments were carried out to select the best medium constituents for evaluating acidogenic and methanogenic activity in the thermophilic range (55°C).

The sludge used in each assay was obtained from a laboratories (5L) anaerobic Stirred Tank Reactor (STR) treating wine-vinasses in the thermophilic range (55°C) by effluent centrifugation at 10,000 g for 15 min. These conditions of centrifugation were selected in order to minimize the VSS (volatile suspended solids concentration) in the supernatant liquid phase following centrifugation, thereby obtaining the maximal sludge concentration.

Two objectives are met by centrifugation; firstly, an amount of biomass within a known specific range of concentration is obtained; and secondly, the different substrates present in the inoculation medium, which could change the values of the initial substrate concentration in the test, are removed. The biomass concentration (Xo) was measured by analysing the volatile suspended solids concentration (gVSS/L) (Clesceri and Greenberg, 1989).

A selected medium containing the principal macro and micro-nutrients (Table 1) were added to the assay reactor. The centrifuged sludge (into a range between 0.15-0.35 for AA and 0.5-1 for MA, expressed in gVSS/L) was added to this solution and then the vial was sealed and flushed for 5 min. with N_2. In the methanogenic test, the gas-meter was connected at this moment. The reactor was immersed in a thermostatic bath for 1 hour. After this time, the specific substrate -

glucose or acetate (with a concentration between 2-3 g/L) -, for the acidogenic or methanogenic test respectively, was added to each assay. Measurements were begun at this moment.

The determination of **acidogenic activity (AA)** was carried out by measuring glucose consumption i.e. by assessing the amount of reducing sugars remaining. The determination of **methanogenic activity (MA)** was carried out by measuring the methane produced from acetate as the methanogenic substrate.

RESULTS AND DISCUSSION

The conditions and procedure for performing the activity assay are described by several authors in the bibliography, but there is no agreement on the composition of the nutrient media. Moreover, the activity of anaerobic biomass in the thermophilic temperature range (optimum 55°C) is not well-known. For this purpose, several experiments were carried out to determine the most suitable media composition, as shown in Table 1, measuring the influence of the following factors: micronutrients (Yeast extract), macronutrients (Nitrogen and Phosphorus sources) and assay media pH (use of buffer solutions - phosphate buffer (pH ~ 7) - bicarbonate buffer (pH ~ 8-9) - and/or neutralization of the assay medium).

A series of experiments was performed to check the influence of the above factors, both separately and in combination, with the methodology described previously. Minimization of the assay duration and reproducibility were used as selection criteria for the best conditions obtained.

A prolonged lag phase was observed in all assays which were done without micronutrient. However, the addition of a micronutrient (yeast extract 0.2 g/L) in both activity tests reduced this phase and, for this reason, the addition was considered necessary. $Na_2S{\cdot}9H_2O$ was used in both cases to maintain a reducing assay medium i.e. with a low redox potential.

Table-1 Media composition (g/L) selected for activity tests.

	AA	MA(*)
$Na_2S \cdot 9\,H_2O$	0.1	0.1
Yeast extract	0.2	0.2
$NaHCO_3$	1	--
K_2HPO_4	--	1
KH_2PO_4	--	2.5
NH_4Cl	--	1
$MgCl_2$	--	0.1

(*) Assay medium must be neutralized before reactor is sealed

The acidogenic activity assay utilizes a bicarbonate buffer that is formed by HCO_3^-, to which 1g/L of $NaHCO_3$ is added, and the CO_2 generated in the medium by the acidogenic biomass. This solution maintains the medium at an alkaline pH which decreases the inhibitory effect that the free acids produced have on the acidogenic biomass (Moletta et al., 1986).

The methanogenic activity test medium is similar to the mineral solution proposed by Valcke and Verstraete (1983). The medium requires the addition of a buffer solution, but in this case, a phosphate buffer (which maintains the pH at close to neutrality) is preferable to a bicarbonate buffer solution. The addition of a macronutrient (sources of

Nitrogen and Phosphorus) was shown to be necessary for the development of the test, since in the tests done without macronutrient the methane generated was less than that which would be expected from acetate removal. The initial neutralization of the medium after the addition of a phosphate buffer assists the development of the methanogenic activity test

In both tests, the activity was defined as the maximum activity attainable. This parameter is the relationship between the maximum rate of consumption of substrate (glucose) or the maximum rate of generation of a product (methane) and the concentration of biomass in gVSS/L.

Activity can be calculated by applying the experimental results to different kinetic models, as Monod kinetic model (Monod 1949; Soto et al., 1993), through a simple linear regression of the experimental results. The tests carried out under these experimental conditions are highly reproducible. An experiment with five identical tests has shown that the standard deviation with respect to the mean maximum activity value is less than 3%, the result of obtained maximum acidogenic activity was 18.30 ±0.5 gGlucose/gVSS·d.

CONCLUSIONS

The principal characteristics of the proposed activity tests are:

- The protocol (equipment and methods) and instruments necessary for performing these tests are not very complex, namely centrifuge, thermostatic bath, UV-VIS-spectrophotometer, etc.
- The tests developed allow the activity of both methanogenic and acidogenic floras present in thermophilic anaerobic reactors to be described.
- The tests permit the measurement of the maximum activity of the different anaerobic microorganism groups in a short space of time - approximately 24 hours in acidogenic assays and 2 days in the case of methanogenic assays. This information, speedily available and highly reproducible, is very useful for the operation and control of an anaerobic reactor. The tests developed can be used to perform standarized inhibition studies.

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