

# Innovative Parameters to Control the Efficiency of Anaerobic Digestion Process

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Anaerobic Digestion (AD) is a complex biological process resulting in the conversion of biodegradable organic matter into biogas and mineralized material. Recently, treating waste technologies based on the AD process have gained a great interest in the field of solid waste disposal as a method coupled with aerobic composting process, aimed at reducing the harmful impact produced on the environment by land-filled untreated organic waste. Moreover, the application of AD for the treatment of organic waste turns into the following further benefits: (i) recover a renewable energy vector (i.e. biogas) as well as a solid material rich in nitrogen (i.e. digestate); (ii) reduce the use of land to dispose waste; (iii) reduce the emissions in the atmosphere of the gases responsible for the global warming. The present paper deals with the experimental determination of operating parameters that are able to control the evolution of AD.

## 1. Introduction

Anaerobic Digestion (AD) is a multi-step, highly dynamic, biological process, which is characterised by several physicochemical and biochemical reactions in sequential and parallel pathways (Figure 1). Different groups of microorganisms are involved, each of them evolved to live in specific environmental conditions and specialized to degrade complex organic matter into simpler end products, mainly CO<sub>2</sub> and CH<sub>4</sub> (Panico et al., 2014).

The complexity of the process makes AD extremely sensitive to changes in environmental conditions, e.g. pH, as a consequence of organic acids production. However, in the last 20 years, the technologies based on AD have gained a growing interest in the field of solid waste treatment, mainly food waste (Ariunbaatar et al., 2014) and in the field of renewable energy, i.e. biogas with a potential calorific power of 10,000 - 20,000 kJ/Nm<sup>3</sup> (Malave et al., 2014).

Although AD are performed by using consolidate technologies, due to the knowledge and expertise recently gained from the widespread use of this process, cases of failure or malfunctioning are still frequent (Labatut & Gooch, 2012). The main reason is usually related to the balance among reactants and products, which is strictly linked to the specific rates of cell growth and substrate consumption shown by different microorganisms taking part to the AD process (Batstone et al., 2002). Indeed, if the concentration of a particular intermediate reaches the homeostatic equilibrium for the given group of microorganisms, the balance is lost (Pontoni et al., 2015). As a consequence, the intermediates start to accumulate thus creating inhibiting conditions (Liotta et al., 2014)(e.g. pH and alkalinity drop) for the activity of the microorganisms that are more sensitive to

environmental conditions changes. Then a gradual reduction of substrate stabilization and biogas production occur and the process experiences underperformance and eventually the failure.

In order to avoid these effects, a correct design of the process and a continuous monitoring of the key parameters - mainly the concentration of intermediate and end products as well as their speciation - are doubtlessly essential tools to obtain high performance and safety.

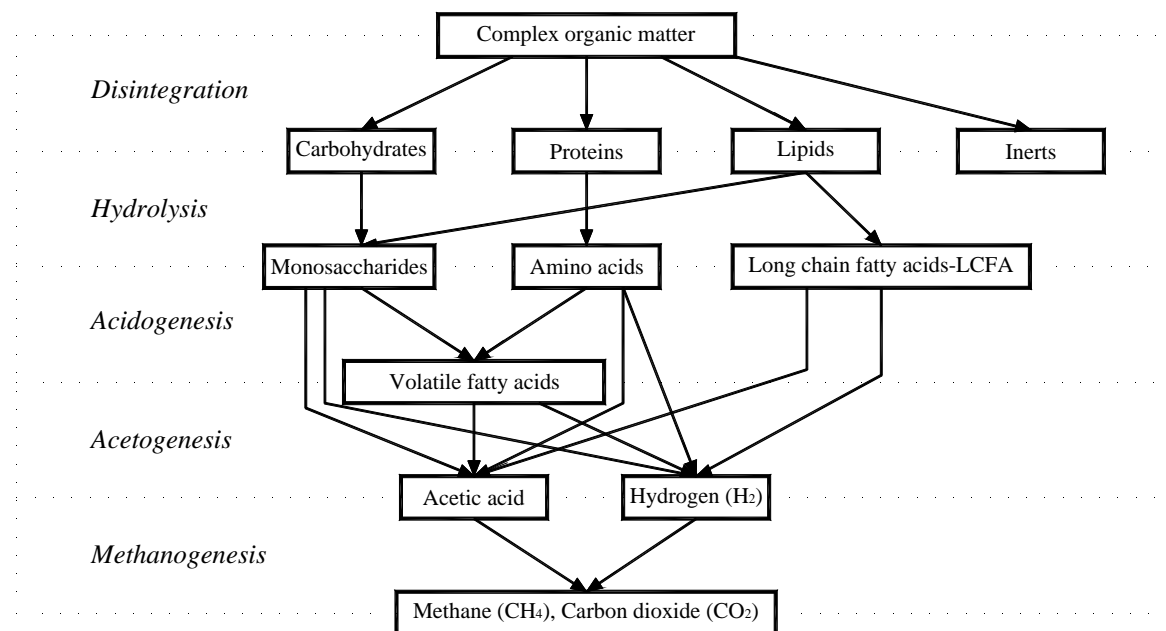


Figure 1: Anaerobic Digestion process flow chart

This paper presents the result of an experimental study conducted on AD process with the specific aim of inducing it to fail and in the meanwhile collecting data concerning the trend of the key parameters that are useful to control the process evolution.

## 2. Materials and methods

### 2.1 Experimental setup

The AD experiments have been performed in a thermal controlled (ADI1025 bioconsolle – ADI1010 biocontroller, Applikon, NL) bench scale 2.2 L anaerobic reactor, made of a vessel with a thermal jacket, provided with an internal mechanical mixer, and hermetically sealed (on the top) by a stainless steel plate. The system has been monitored in real-time by a control panel implemented in LabVIEW (Applikon, NL).

The anaerobic sludge collected from the anaerobic digester in real scale, treating dairy waste produced by a mozzarella cheese factory located in Capaccio (Salerno, Italy) and thermally stored at 4° C. The sludge characterized by total solids (TS) and volatile solids (VS) content of 2.49 ±0.08 % (m/m on wet mass) and 66.9 ±0.7 % (m/m on dry mass) was used to fill the reactor as inoculum (Kheiredine et al., 2014a).

A liquid solution containing 1 g of glucose in 40 mL of demineralized water was used to feed the reactor, daily. No macro as well as micro nutrients were added to the feeding solution with the aim of gradually inducing the process to fail and acquiring for the key parameters controlling process.

A working volume of 1.7 L was set for the experiment and the reactor was operated in semi-continuous mode. Simultaneously to feeding operation, an equal flow of digestate was discharged.

Temperature, pH and dissolved oxygen content of the digesting mixture were continuously monitored by specific probes immersed in the liquid bulk of the reactor. Daily production of methane was measured with a volumetric system hydraulically connected with the reactor head space as described in Esposito et al. (2012). Table 1 summarizes the main operational parameters of the completely stirred tank (CST) reactor used.

Total and carbonate alkalinity, Volatile Fatty Acids (VFAs), pH and Total Ammonia Nitrogen (TAN) were accurately monitored during all the experimental time in order to determine those key parameters that better than others can be used to control the anaerobic process efficiency and reliability.

Table 1: Operational parameters of the CSTR anaerobic digester.

Vol (L)	HRT (d)	OLR (g <sub>COD</sub> d <sup>-1</sup> )	Temperature (°C)	Avg methane yield (NmL/gC <sub>6</sub> H <sub>12</sub> O <sub>6</sub> )	Avg max pressure in the headspace (bar)
1.70	42.50	1.07 ± 0.11	37.0 ± 0.5	337 ± 69	2.51 ± 0.14

## 2.2 Analytical methods

TS and VS content in the inoculum and digestate were measured by a gravimetric method according to EPA standard method N° 1684. Before being analyzed, the liquid fraction of digestate was extracted by centrifugation by using a Micro 22R (Hettich, D) centrifuge. TAN was determined by steam distillation followed by titration by a semi-automated distillation unit (UDK 132, VELP, IT). Total and carbonate alkalinity and VFAs were determined by titration according to Anderson and Yang (1992). The titrations were performed by using an automated titrator (TTT80, Radiometer Copenhagen, F). The speciation of VFAs was obtained by extracting the VFAs through solid phase micro-extraction of the head space (HS-SPME) technique using a modified version of the method proposed by Abalos et al. (2000). In detail, a 2.5 mL clean SPME vial sealed with silicon septum (Supelco, USA) was filled with 100 mg of sulphaniilic acid (Carlo-Erba, IT). In sequence, 200 µl of sample and 50 µL of a 50 mg/L solution of 3.3 Dimethyl butanoic acid solution as internal standard were added. The septum was then punched by the needle of the SPME fiber and the system was kept to interact with the retractile fiber (85 µm Polyacrylate, SUPELCO, USA) at 80°C for 25 min. Once the extraction time was ended, the fiber was injected into the GC-MS (Agilent 6850, coupled with Agilent 5973 MS detector) inlet liner set at 140°C, where the desorption of the analytes took place. The fiber was selected on the basis of its high performance for those specific extraction conditions. Through seven experimental points the calibration curves were drawn on the basis of response factors for each of the VFAs analyzed using standard solutions with a VFAs concentration ranging from 0.25 to 100 mg/L.

Sulphanilic acid provided the solution with a stable final pH of 3.4 and was preferred to the saturated NaCl/HCl system proposed by Abalos et al. (2000). The higher volatility of HCl compared with sulphaniilic acid leads the HCl to be responsible for a faster deterioration at high temperature of the SPME fiber. For this reason the fibers showed a decrease of extracting performances already after several tens of extractions/injection cycles. Extraction operations at temperature lower than 80°C are hence recommended (Abalos et al., 2000) when HCl is used to increase volatility. This limitation causes a loss of measurement method sensitivity and/or an increase of the extraction time. The use of sulphaniilic acid gave to the method a higher level of sensitivity and reproducibility for a greater number of cycles also when the operating temperature was set at 80°C

Moreover the zwitterion behavior of sulphaniilic acid guaranteed that it did not interfere with the analysis due to its low volatility that prevented its adsorption on the fiber and also coordinated a high number of water molecules favoring a good salting-out effect for the volatile analytes. The column was a 30 m Nukol (SUPELCO, USA) fused silica capillary column with 0.25 mm inner diameter and 0.25µm film thickness.

Total VFAs (TVFAs) were calculated by direct titration of the samples and by the sum of the concentrations of the single VFAs obtained from the GC-MS analysis.

## 3. Results and discussions

The reactor was operating for 75 d. Trends of the key parameters controlling the anaerobic process are shown in Figures 2-6. In Figure 2, the daily methane production is compared with the pH of the digesting mixture. It is easy to see in the graph the sequence of three operating phases: a first phase lasted approximately 35 d where the reactor ran regularly and properly almost showing a steady state condition. From the day 35, it can be noticed a slow decrease of pH and methane production that progressively become faster. The failure occurs at day 62. From day 62 the reactor was not be capable to recover itself.

In the first 35 days the methane production was pretty constant around 400 ml/d and pH fluctuated around 7.4, showing small oscillations due to the semi continuous feeding system. After each feeding, actually, a sharp drop of pH is observed caused by the acidogens' activity, immediately recovered by the methanogens' activity that consumes organic acids and produce methane. As the reactor was not provided with any extra source of alkalinity and the bio-methanation process requires a small amount of alkalinity to perform, the pH showed a slow decreasing trend as long as the system was far to fail. From day 35 the pH started not to recover the previous value after each feeding operation and dropped around 6.8 at day 62. Simultaneously, as expected because the methanogens are really sensitive to pH changes (Kheiredine et al., 2014b), the methane production progressively decreased down to reach a value of only 20 mL/d. Several attempts, described in detail in the next lines, were done to recover the reactor however with no success.

Observing Figure 3, both the total alkalinity and the TAN showed a decreasing trend during all the reactor running: about alkalinity, the explanation for its decrease has been already given above, whereas, ammonia nitrogen decreased cause of the dilution process due to the feeding with a solution with no ammonia content.

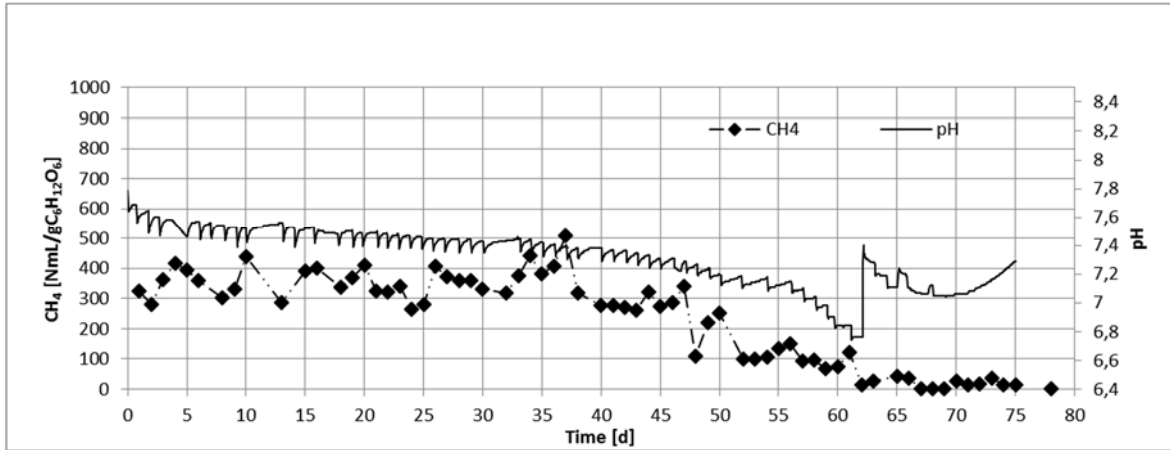


Figure 2: Methane production and pH monitoring

From the analysis of Figure 3, the first attempt to recover the process was to restore the initial alkalinity by adding to the feeding solution an opportune amount of sodium hydrogen carbonate. After some days, as no increase in methane production was observed, at day 65, an amount (3.6 mL) of 30 %  $\text{NH}_3$  solution was added to restore the initial ammonia content in the reactor, but still no production of methane was achieved, neither after adding (day 68) a solution of nutrients (8 g/L of  $\text{NaHCO}_3$ , 5 g/L of  $\text{NH}_4\text{Cl}$ , 80 mg/L of  $\text{KH}_2\text{PO}_4$ ) including trace metals (Ni, Mo, Co, Cu, Zn, Fe, Mn). This result proves that once failed, the system can experience a quenching of the methanogenic activity that, if not irreversible, is very difficult to be remediated. Furthermore the observed irreversible failure of the reactor learns how much delicate and therefore necessary is the continuous and accurate monitoring of the anaerobic digesters and the right choice of the key parameters to be controlled during the process. One of them is doubtlessly the pH. The only pH is not enough to prevent digesters failure. From the discussion of the results in Figure 4 it will result that pH coupled with the ratio between VFAs and total carbonate alkalinity concentration (FOS/TAC ratio) could provide the system with useful information that can help to successfully prevent the failure of the anaerobic digester.

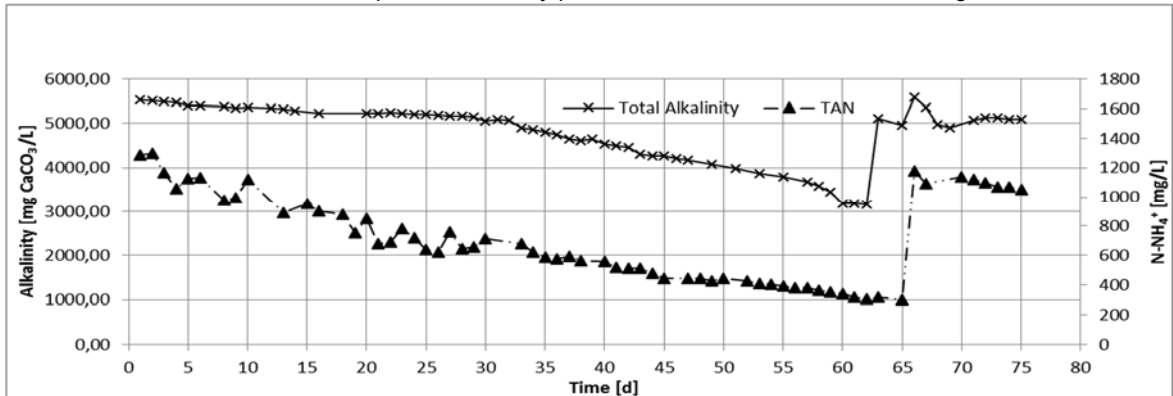


Figure 3: Alkalinity and Total Ammonia Nitrogen monitoring

Figure 4 shows the trend of the FOS/TAC ratio during the experiment comparing it with the pH trend. From Figure 3 it can be easily noticed that the FOS/TAC ratio showed low and constant values, around 0.15 during the first 35 d of the experiment when the reactor running was approximately stable. the FOS/TAC showed at day 44 a slight increase that grows exponentially on the next days until the day 63 when alkalinity was artificially added. It is worth to notice that at day 44 the reactor was still regularly producing methane (almost 300 mL/d) and the pH although around 7.2, was still in a range that does not affect seriously the anaerobic digestion performance. At day 44, 5 d earlier than the system experienced a significant drop in methane production and pH value, very likely the reactor was still in the condition to be recovered. Therefore an accurate monitoring of FOS/TAC ratio can provide the anaerobic digestion process with a warning level that alerts on time when the digester starts to experience an irreversible failure event (Ariunbaatar et al.,

2015). The increase of the FOS/TAC ratio is tightly related to the decrease of alkalinity previously discussed as well as to the accumulation of VFAs in the reactor.

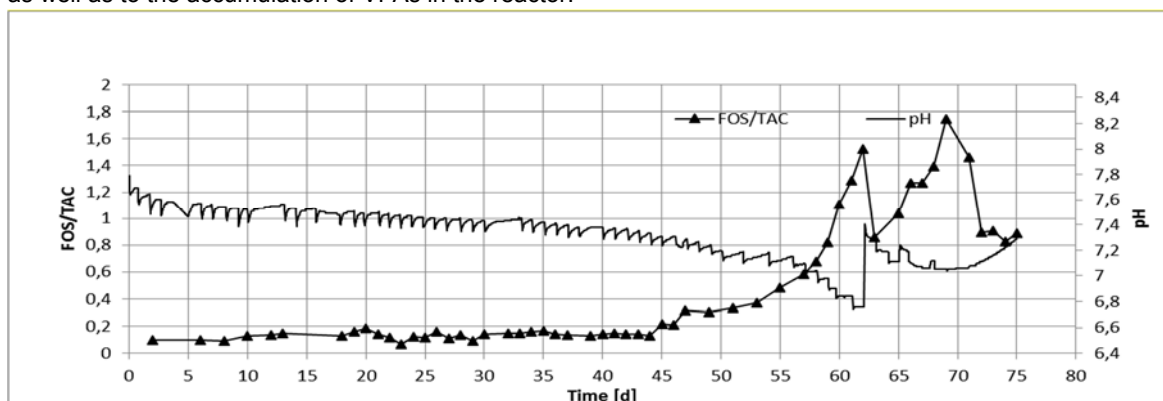


Figure 4: Trend of FOS/TAC ratio compared to pH

Figure 5 reports the concentration of TVFAs (Figure 5a) as well as the VFAs speciation (Figure 5b). From graphs in Figure 4 it can be observed that the VFAs accumulation that caused the FOS/TAC ratio increase, was mostly due to an accumulation of propionic acid that reached extremely high values (more than 2,500 mg/L) that did not decrease neither when alkalinity or ammonia were artificially added to the reactor at day 62 and 65. Around day 55 the system started to experience a progressive accumulation of both acetic and butyric acids that proves the total inhibition of methanogens' activity, with no bio-methane production as a result. Adding nutrients on day 68 resulted in the reduction of the acetic acid concentration as well as the consumption of a part of the propionic acid. Anyway no more methane production was observed time running, whereas the production of longer chain VFAs (butyric, valeric, isovaleric acids) was still evident. It is reasonable to justify this overcome assuming the existence of some other metabolic pathways irreversibly activated in competition with the methanogenesis.

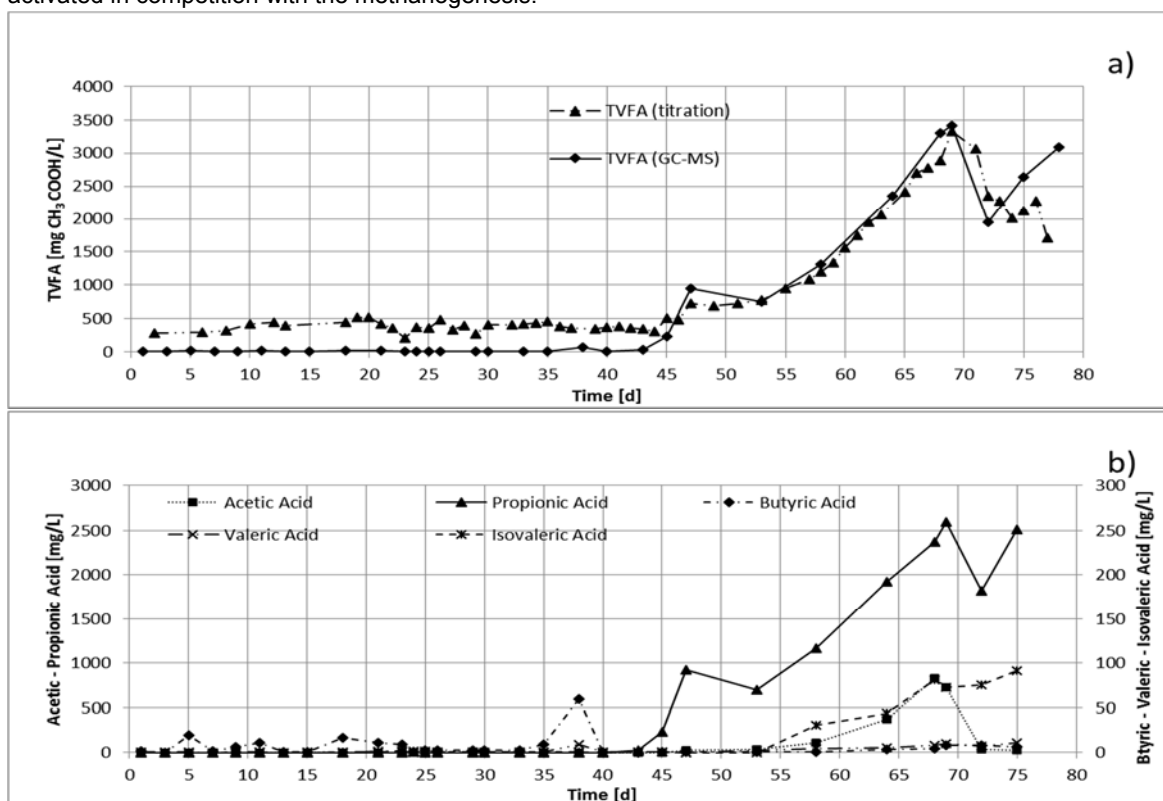


Figure 5: TVFAs concentration (a) and VFAs speciation (b)

#### 4. Conclusions

The handling of the anaerobic digestion process is extremely delicate. When a failure event occurs it is really difficult to recover the system, therefore it could be very useful to know in advance when the digester starts to experience underperformance that could lead the process to fail. This research has showed that coupling the measure of pH with FOS/TAC ratio with speciation of VFAs makes possible to manage an anaerobic digester reducing the risk for its failure. Moreover the suggested parameters are of easy and cheap applicability to full scale digesters.

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