Replication of experiments in anaerobic digestion: simpler, greater and easier than you had ever hoped

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Abstract
Reliability of results in science is done by replication of experiments, but it is often expensive and labour intensive. Indeed, operating anaerobic digesters in a continuous mode requires some experience and a lot of manpower. The number of anaerobic digesters that can be handled in parallel is therefore limited. In practice, experiments are often repeated several times, starting always with a fresh inoculum. One approach is to store and to restore the activity of the same inoculum, with no guarantee on the preservation of the functioning. Unfortunately, there is no consensus on any standardized strategy for the long-term preservation of complex ecosystems. Here, we present a multiplexed chemostat system that minimizes costs and manpower for operating several anaerobic digesters in parallel, and present a first application of the system. Up to thirty reactors may be operated by a single person. This system may unlock ecological studies as well as process optimization in the context of anaerobic digestion, where several conditions need to be tested in parallel. The vocation of this device is to make it available to the scientific community.

Keywords
chemostat; process optimization; replication; microbial ecology; microbial engineering

INTRODUCTION

Lack of replication is a recurring problem in experimental work [Prosser, 2010]. Compared to natural ecosystems, replication in the laboratory is relatively easy, especially when using batch experiments. It is indeed a popular strategy for the initial screening of the optimal condition, when associated with an appropriate experimental design. Commercial devices are also already available for anaerobic digestion, for example the AMPTS (Automatic Methane Potential Test System, Bioprocess Control, Sweden).

Results obtained from batch experiments may not be relevant in an ecological context while chemostats are typically better suited for studying long-term behaviours of microbial communities and their functioning. However, the operation in continuous mode of several bioreactors at the same time requires a significant amount of manpower, increases significantly the costs of the operation and the complexity of the experimental set-up. It is especially true for anaerobic digestion since we are dealing with slowly growing microorganisms where typical experiments may last for several months. Some prototypes of continuous culture are under development [Matteau *et al*., 2015] but no device is currently commercially available for running several anaerobic digesters in parallel with enough flexibility and affordable price.

One alternative of running several anaerobic digesters in parallel is to store and preserve an inoculum over time, and then revitalizes the biomass to restore its activity when necessary [Hagen *et al*., 2015; Kerckhof *et al*., 2014]. Unfortunately, there is no consensus on a standardized strategy for the long-term preservation of the functionality of complex ecosystems, despite the importance of the choice of the inoculum shown in several studies with anaerobic bioprocesses [Raposo *et al*., 2011; Rafrafi *et al.*, 2013].

Here, we present a new multiplexed chemostat system that minimizes manpower for operating several anaerobic digesters in parallel. Up to thirty reactors may be operated at a time by a single person. The working volume, dilution rate and temperature can be tuned as desired. Sterile feeding, biomass wasting, quantification of biogas production and degassing are fully automated. The versatility of this new multiplexed chemostat system makes it a feasible option for a wide range of experiments. We present here a first application of this system with twelve bioreactors on the response of three anaerobic sludge origins to a fluctuation of the organic loading rate, using four replicated anaerobic digesters over nine weeks.

MATERIALS AND METHODS

Design of the multiplexed chemostats

One module is composed of six units of automated chemostats. Automated feeding, withdrawal and degassing are done by peristaltic pumps with stepping motors controlled by a computer. A heating block controls the temperature above room temperature. All the parts in contact with the reactor interior can be autoclaved for sterile operating conditions.

First application

Three different sludge origins were taken from pilot-scale anaerobic digesters fed either with readily biodegradable substrates (grass and carrots), slowly biodegradable substrates (manure and dung) or intermediate (grass and manure). The three types of sludge were inoculated in four replicated bioreactors each. The anaerobic digesters were fed in continuous mode under mesophilic conditions (37°C) for nine weeks with a hydraulic retention time of 15 days and an organic loading rate of 1.33 gCOD/L.d, except for weeks 4 to 6 where the organic loading rate was lowered to 0.67 gCOD/L.d. Biogas production was recorded on line and expressed on a weekly basis in $mL/gCOD_{added}$. Several operating parameters, like pH, biogas composition, volatile fatty acids or volatile solids, were measured on a weekly basis.

RESULTS AND DISCUSSION

Range of operating conditions of the multiplexed chemostats

A module hosts six chemostats with the flexibility for independent automated operating conditions (Figure 1). Up to 30 chemostats can be operated by one person with a minimum of maintenance. Many parameters can be tuned as desired: the working volume [ranging from 50 to 200 mL], the dilution rate [from batch mode to a complete replacement of the reactor volume below 20 min], the pressure [ranging from 0 to 3.4 bars with a defined interval, 0.2 by default] and. the temperature [from room temperature to 55°C].

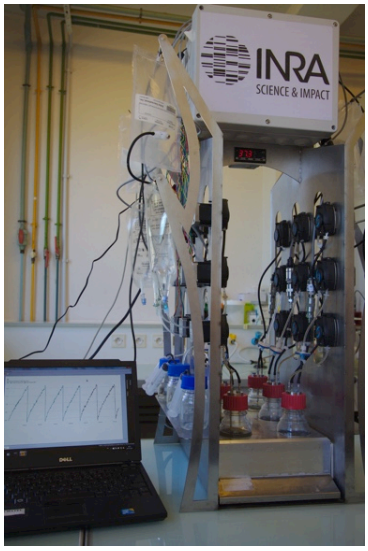

Figure 1: Module of six chemostats with the computer that controls the pumps and collects the data.

First application of the multiplexed chemostats

Twelve anaerobic digesters were operated in parallel with a minimum of maintenance requirements. Biogas was recorded over time by pressure measurements, and biogas production rates reported as a function of sludge origins and time (Figure 2). Despite variability among replicates, differences in biogas production rates have been measured over time and according to sludge origins (Kruskal Wallis tests). For example, the sludge originally fed with readily biodegradable substrates (grass and carrots) decreased its performance over time and was very sensitive to any change in organic loading rate. The sludge adapted to degrade a more complex substrate with a blend of readily and slowly biodegradable organic matter (grass and manure) was the only one that recovered its initial activity after two variations in organic loading rate.

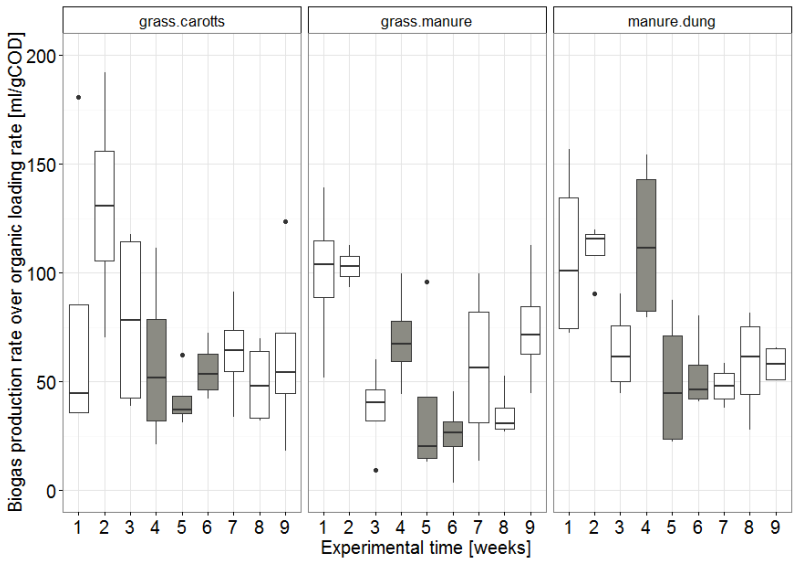

Figure 2: Boxplot of four replicates showing biogas production rates obtained for the three different sludge origins over nine weeks. White boxes stand for the high organic loading rate condition (1.33 gCOD/L.d) and grey boxes stand for the low organic loading rate condition (0.67 gCOD/L.d).

While differences in the biogas production rates could have been shown according to the origin of the sludge (Figure 2), similar behaviours were observed in terms of volatile solids (Figure 3) and

volatile fatty acids (data not shown). Significant differences in biogas (Figure 2) accounted for the specific activity of microbial communities since total biomass did not differ significantly between sludge (Figure 3).

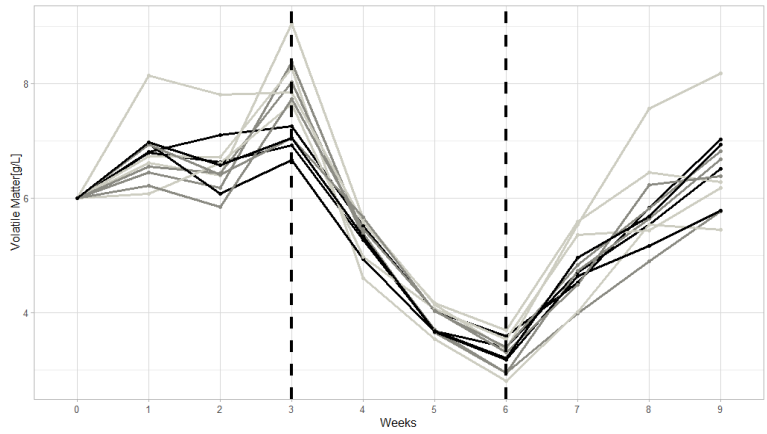

Figure 3: Dynamics of volatile solids (g/L) for the twelve anaerobic digesters over nine weeks. High organic loading rate conditions (1.33 gCOD/L.d) were applied at the beginning and at the end of the experiment, and low organic loading rate conditions (0.67 gCOD/L.d) were applied in the middle.

CONCLUSION AND FUTURE DIRECTIONS

We developed a simple, automated multiplexed lab-scale anaerobic chemostat system. This new device was designed to be very flexible (sterility, hydraulic retention time, organic loading rate, working volume, temperature, etc.). We believe this new experimental device will unlock research on anaerobic digestion, often impaired by our ability to handle several bioreactors in parallel. One significant scientific advance is that the same sludge can be used repetitively under strictly controlled conditions. This work opens new research avenues for a better understanding of the functioning of microbial communities in the context of anaerobic digestion.

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