Application of F420 diagnostic as operational support for industrial anaerobic reactors

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Abstract
Discovered 35 years ago, the microbiological observation of methanogens with F420 fluorescence remained a long time a simple and limited laboratory tool. Following repeated requests from operators of biogas plants, this observation protocol has been adapted to become an easy and fast diagnostic tool. These situations are most often diagnostics of biological activity after accidental passage of an inhibitor or toxic compound in the reactor, assessment of the quality of an anaerobic biomass before seeding a new reactor (density, diversity), start-up monitoring of an anaerobic reactor, or long-term evolution of biomass in the reactor and prevention of performance decrease.
Since 2008, the feedback has been capitalized and is returned here through two case studies. In one case, the F420 observation has allowed a better understanding of the causes of a toxicity accident in an anaerobic fluidized bed reactor in food industry. The F420 diagnostic has permitted to make the good choice by applying an adapted biomass seeding of the reactor, and by discussing with the industrial customer how to limit the toxic parameters in the wastewater. In the second case, F420 has permitted to confirm the degranulation factors in an EGSB reactor operated in a paper industry. Further to the F420 analysis results, the operator decided to investigate the root causes of degranulation as part of his on-going action plan.

Keywords
F420, anaerobic reactor, operational support, microbiological analysis, methanogens diversity, methanogens density, multilayers structure

INTRODUCTION

Operators of anaerobic reactors facing biological problems, for which control parameters conventionally used on site do not provide satisfactory answers, frequently approach the technical experts of Veolia. It is often required to check the status of the anaerobic biomass in the reactor, as methanogenic archaea. As first approach, a simple "binary" answer (living or dead cells) is sufficient to operators, as well as some information on diversity, or density. It is important to provide operators quick response, almost instantaneously, given the constraints of an industrial site.

To address this problem, a technique for microscopic observation of fresh anaerobic sludge was identified from ancient literature (Mink *et al.*, 1977; Doddema *et al.*, 1978): the detection of methanogens by F420, based on the autofluorescence property of coenzyme F420, electron carrier involved in redox reactions in methanogens. It has the characteristic to emit a blue fluorescence when excited at a wavelength of 420 nm. This specificity allows visualizing methanogens in environmental samples without prior labeling.

Veolia has implemented this technique successfully since October 2008. In an industrial site in the South of France where Veolia Water operates a "fluidized bed" digester, the diagnosis was used to select, from different sources, the most appropriate biomass for a fast start-up of the reactor.

Thereafter, several other industrial and municipal sites have requested this type of diagnosis. Given the increase in demand since 2009, it became necessary to develop this service in the Technical Assistance team of VERI with the help of microbiologists. In 2010, technical assistance experiences with this tool showed the quickness and efficiency of this application. Among the case studies from R & D and operational expertise developed on industrial biogas plants, two examples of applications using this tool are presented below.

MATERIALS & METHODS

Fresh samples were taken and stored into flasks filled up to the top to avoid contact with oxygen, and kept at 4°C until shipment into an icebox. Microscopic observations were performed on fresh sample, using an epifluorescence microscope Olympus BX51 equipped with excitation filter U-D425/40X 405-445 nm and emission filter U-E455LPV2. CCD Camera and Cell software were used to take photographs.

RESULTS & DISCUSSION

Case 1: Washing and viability loss of an anaerobic biofilm

A fluidized bed anaerobic reactor treating industrial effluents had significant decrease in the COD (chemical oxygen demand) removal efficiency, and increase of VFA (volatile fatty acids) concentration at the reactor outlet during summer 2011. Presence of inhibitory or toxic compounds in industrial effluents was suspected.

F420 analysis was implemented to check an impact on the biofilm at different heights of the digester. Macroscopic appearance of samples revealed four phases of settling (supernatant, interphase, granules, and inorganic support), all were observed with F420.

The inorganic support from height 1 had incomplete colonization, with about 1/3 supports colonized by methanogens. After uncoupling the biomass from its support, only few round-shaped archaea were observed in the flocculent sludge. This confirmed the low density and diversity of methanogens, with a ratio of methanogens / total biomass estimated below 0.1%.

F420 observations on samples from heights 2, 3 and 4 showed similar colonization profiles, with a higher rate than in height 1 (Fig.1A). After uncoupling, only one methanogenic type corresponding to short bacilli in clusters (Fig.1C) appeared fluorescent. This morphology was not present at height 1. An overlap with visible light distinguished many rods long and straight (15-20µm) non-fluorescent (Fig.1B). Only *Methanosaeta*-like type has this typical morphology. With no fluorescence in the 3 heights, these cells could be methanogens having lost any viability.

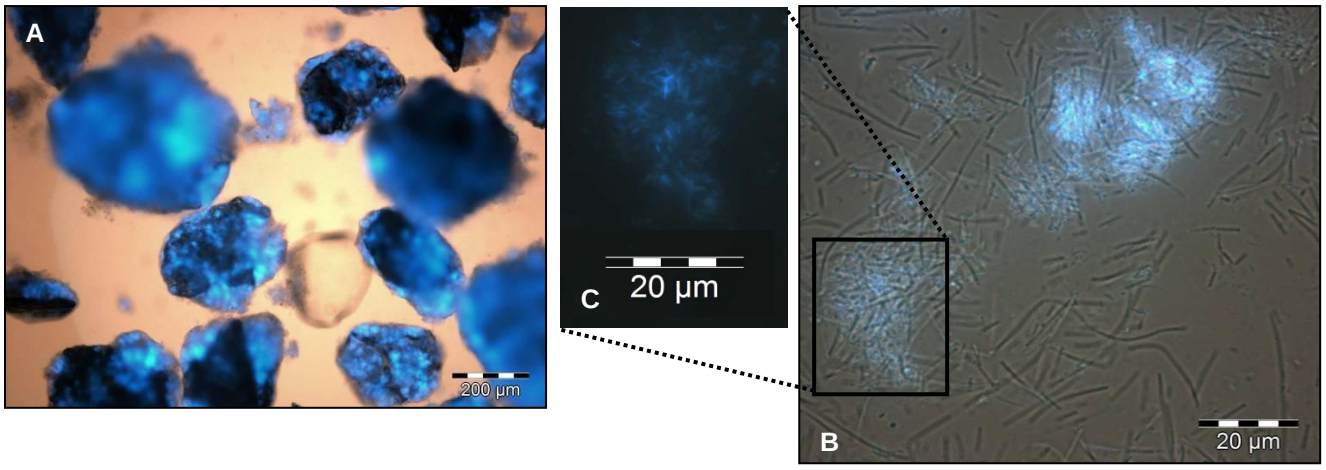

Figure 1. A. Inorganic support from height 3 observed at 420nm: methanogens cells (blue), support (grey-black) (X100). **B.** Methanogen biomass uncoupled from support of height 3 observed under visible light and 420nm (x1000): non-fluorescents straight rods *Methanosaeta*-like (grey), short fluorescents bacilli (blue). **C.** Short bacilli methanogen observed at 420nm (X1000).

These three heights had the particularity of having a "flocculent" interphase after settling. This fraction, corresponding to biomass detached from the mineral substrate, was observed by microscopy. All flocs showed a blue fluorescence typical of methanogens corresponding to that attached to the mineral substrate, namely short bacilli in clusters. The non-fluorescent

Methanosaeta-like was observed on the inorganic support. These observations on biomass of the interphase confirmed the hypothesis of methanogens unhooking from the inorganic support.

Dysfunctioning has reached viability of methanogenic biomass, causing loss of viability of a large methanogenic fraction responsible for degradation of a main VFA: acetate. The integrity of the biomass fixed on the inorganic support was reduced by unhooking in the three upper heights. The support being abnormally distributed to the top of the reactor, nearly 60% of supports (heights 2, 3 and 4) was affected by this unhooking phenomenon and loss of a methanogens fraction. The remaining 40% corresponded to poorly colonized supports (height 1). The decrease in acetoclastic methanogenic populations can be correlated with the evolution of VFA in the digester output (Figure 2). There is a fairly sharp increase in the ratio of VFA and VFA/COD from 25 July 2011. This disequilibrium remains constant throughout August and results in a progressive decrease of the COD removal efficiency. Given these curves, we assume that this acidification event began to disrupt the methanogenic activity in late July and has contributed to the washing of mineral surfaces a

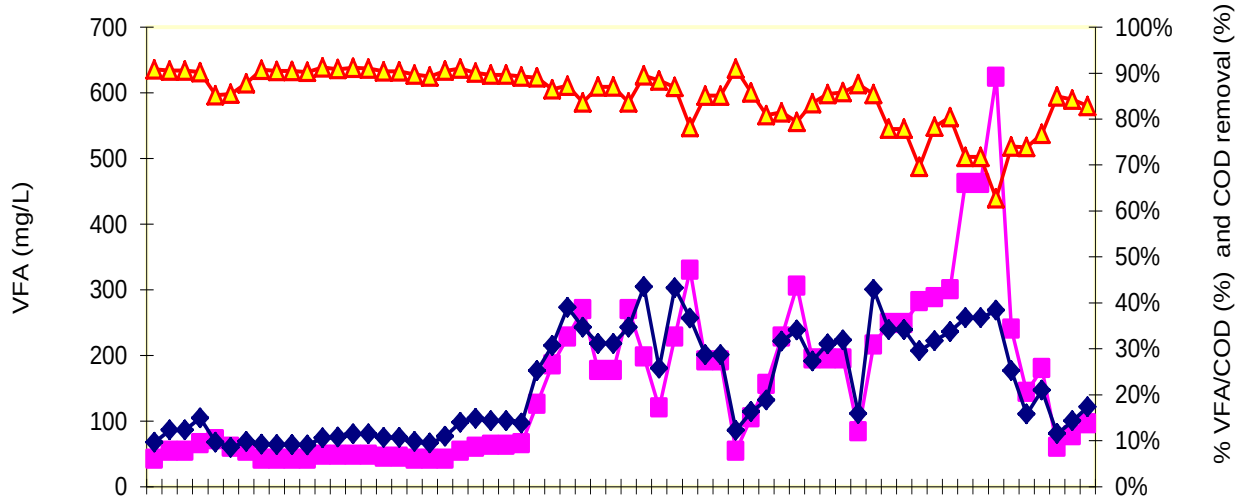

Figure 2. Volatile Fatty Acids concentration (VFA), Acidification rate and COD removal in the outlet of the reactor during dysfunctioning phase.

Following F420 diagnostic, operators concluded that the mineral support was too much washed. Natural growth of anaerobic biomass would be too long. The digester was seeded with an external suitable biomass to quickly colonize supports. F420 demonstrated its efficiency to help the operators in the choice of the optimal solution to recover to nominal capacity of the reactor.

Case 2: Degranulation in an EGSB reactor (Expanded Granular Sludge Bed)

An EGSB of an industrial WWTP in England showing significant granule losses has been a good illustration of combined approach: macroscopic and microscopic observations. It allows the identification of degranulation phenomena and variable colonization of methanogens in granules.

A too high acidification rate at the inlet of the reactor was suspected to cause degranulation. Three samples were then taken at different heights of the reactor to evaluate the impact of acidification. Several phases of settling were characterized with a binocular microscope: supernatant interface "cottony", granules, and remains (Figure 3). It clearly showed a strong heterogeneity in sizes and colors of granules, and numerous remains indicating significant damage.

In addition, cross-section views showed that only few granules had a multilayer structure typical of stable granules. The three samples showed same heterogeneous pattern of granules, illustrating an important degradation phenomenon.

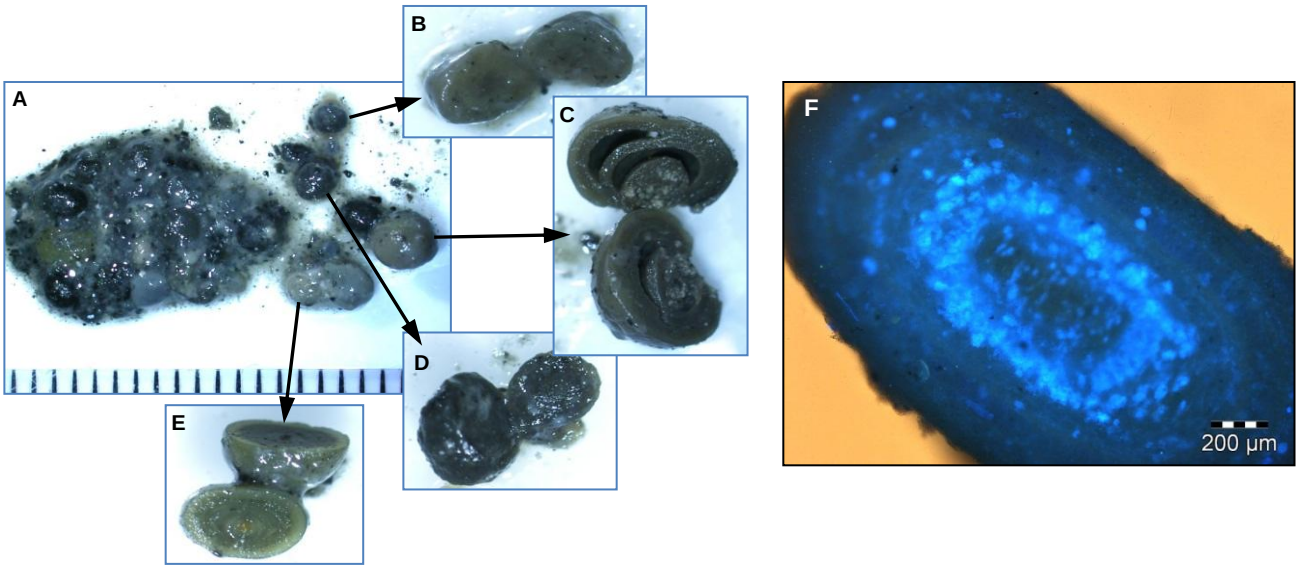

Figure 3. A. Heterogeneous granules in bottom of reactor: remains, granules with different sizes and colors. **B to** Cross **E.** sectional of granules showing multilayers (E and C) or not (B and D). **F**. Observation at 420nm of a whole yellow granule in cross sectional: clusters of methanogens cocci (blue) (x100).

The multilayer structure is a spatial organization of microbial communities involved in anaerobic digestion. Central core is rich in methanogens mainly *Methanobacterium* or *Methanosaeta* and surrounded by layers of bacteria: hydrophobic acid-producers and cells involved in hydrogen cycle (Mc Leod *et al.* 1990). This stable structure promotes production and release of biogas. Settling of granules is thus favored, unlike unstructured pellets with rich acid-producing bacteria and irregular outer surface with many cracks. Thanks to their highly regular surfaces and multilayer structure, the yellow granules (Figure 3E) do not seem to undergo degranulation. Additional observations at low magnification have shown such a spatial organization of methanogens in the core of the yellow granule (Figure 3F).

The fluorescence of methanogens in all samples showed colonization by specific methanogens according to the granules and allowed to evaluate a density of active methanogens around 50-60% in the granules. Cellular remains surrounding granules and interphase have same characteristics in terms of methanogenic diversity and density, confirming degranulation.

Confirmation of degranulation by analyzing F420 has enabled operators to investigate the root causes of this phenomenon as part of their on-going action plan.

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