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## Modelling hydrolysis: simultaneous versus sequential biodegradation of the hydrolysable fractions

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### Abstract

This paper is a discussion proposal of structural model of organic matter hydrolysis (in aerobic and anaerobic bioprocesses) based on laboratory and model results. Indeed, the hydrolysis mechanism is mechanistically not well understood. Consequently, models are sometimes not appropriate to describe experimental results. Where there are multiple fractions, a model that considers simultaneous degradation of the substrates may not have the resolution to separate the different kinetics. In this paper, we assess sequential extractions to evaluate this as an alternative to simultaneous analysis.

### Keywords

Modelling; ADM1; hydrolysis; organic matter; fractionation

## INTRODUCTION

Hydrolysis is the breakdown of a polymer into monomers by addition of water molecules (Brock and Madigan, 1991). In biodegradation of organic residues like wastewater, the processes of hydrolysis is used to refer to all mechanisms that make slowly biodegradable substrate available for bacterial growth (Gujer et al., 1999). In mathematical models, the process of hydrolysis must be adequately described to be able to predict spatial and temporal availability of organic substrate for nutrient removal processes (Morgenroth et al., 2002).

However, hydrolysis kinetics in waste treatment applications are not well understood (Morgenroth et al., 2002), and first order processes are applied as an aggregate approximation (Eastman and Ferguson, 1981). This is true in aerobic and anaerobic bioprocess treatment. Since the first developed activated sludge models (see, for instance, Henze et al., 1987), the hydrolysis concept has been challenged, but with 1st order processes generally dominating in application due to difficulties in identifying higher order models. Modelling oxygen uptake rate (OUR) or methane production rate (MPR) curves often do not identify underlying kinetics due to a lack of resolution.

Morgenroth et al. (2002) presented four different concepts of hydrolysis modelling: (1) dominance of a single substrate reaction (Jones, 1971; Dold et al., 1980), (2) sequential adsorption and reaction, (3) parallel reaction (i.e. simultaneous) with addition of several

organic matter fractions (Sollfrank and Gujer, 1991; Lagarde *et al.*, 2005; Orhon *et al.*, 1998), (4) sequential reaction (Bjerre, 1996; Confer and Logan, 1997; Lagarde *et al.*, 2005; Spérandio *et al.*, 2000). While it is possible to evaluate multiple fractions simultaneously in a single batch test (Wang *et al.* 2013), this is only effective when the fractions have very different kinetics. Some studies proposed solutions to better explain it such as particule breakup models (Dimock *et al.*, 2006; Yasui *et al.*, 2008) based on particle size characterization.

Indeed, from a common conclusion that the hydrolysis rate decreases when particle size increases (Balmat, 1957), particle breakup models (Dimock *et al.*, 2006; Yasui *et al.*, 2008) and shrinking particle models (Sanders *et al.*, 2000) have been utilised. For example, Sanders *et al.* (2000) used surface-based kinetics, where the rate of hydrolysis was proportional to the available surface area of slowly biodegradable organic matter. This particle breakup can result in an increase of the available surface area as hydrolysis progresses (Dimock *et al.*, 2006).

In the case of simultaneous and sequential concepts, the biodegradable fraction is often fractionated according several methods. For example, Ekama and Marais (1979) divided the wastewater into two biodegradable fractions that are degraded at two different rates, a readily biodegradable fraction consisting mainly of soluble organic matter and a slowly biodegradable fraction that consists of large molecules, colloids and particles that have to be hydrolyzed before degradation. The distinction between these two fractions was also determined by experimental biological response (Ekama *et al.*, 1986; Sperandio *et al.* 2000). This was also the case for anaerobic biodegradation tests (Yasui *et al.*, 2006, Mottet *et al.*, 2013).

Indeed, research on hydrolysis is based on four experimental approaches quantifying (1) Enzymes, (2) hydrolytic intermediates, (3) bulk parameters, or (4) bacterial kinetics (Morgenroth *et al.*, 2002). According to the authors, the first two approaches allow to study mechanisms involved in hydrolysis but restricted to specific substrates (e.g. starch). The latter two approaches allow evaluating the overall processes but are not the specific mechanisms involved in the hydrolysis process. The challenge is to find one experimental way to deeply study the mechanisms of hydrolysis.

If we consider the definition of hydrolysis, it refers to the breakdown of organic substrate into smaller products that can subsequently be taken up and biodegraded by bacteria. This definition considers two majors concepts: bioaccessibility and biodegradability.

Due to the complex organization of some organic residues, the bioaccessibility is defined as the possible access to the molecule. It can depend on the process duration, the hydrolytic activity or the pre-treatment applied before the treatment. Then, the bioaccessible fractions become bioavailable. Molecules with a weight below 1000 Da can pass through the cell wall (Aquino, *et al.*, 2008). Finally, the biodegradable fraction is the organic matter bioavailable consumed by the microorganisms.

While biodegradability tests are well known (Angelidaki *et al.*, 2004), characterization of the bioaccessibility remains a challenge. Recently, a new promising methodology of organic matter characterization has been successfully developed to describe the organic matter bioaccessibility of organic residues (Jimenez *et al.*, 2015a) and predict their biodegradability. This methodology is able to provide new organic matter variables for particular organic matter. Jimenez *et al.* (2015b) showed that they could be successfully used in a modified ADM1 model to predict biogas performances and digestate quality of a anaerobic digester fed by sludge.

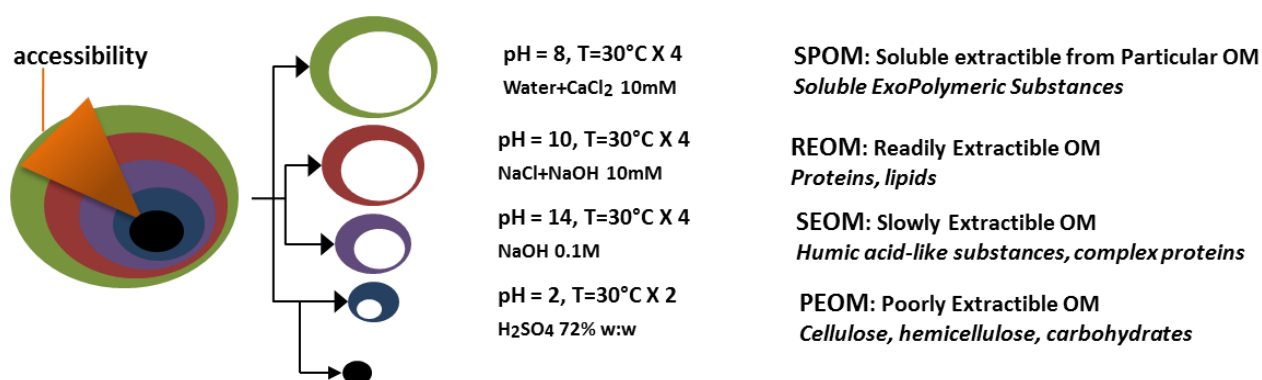
The objective of this paper is to challenge the classical parallel concept of multi-substrates hydrolysis and to provide a methodology applicable to different substrates. The use of a new fractionation methodology, describing accessibility, could indeed help to better understand and describe this phenomenon. The fractionation developed is used now as anaerobic digestion model input variables for a plant wide organic wastes treatment objective. However, some questions and hypothesis were needed to properly calibrate the model and predict the MPR. A discussion about conceptual approach of organic matter hydrolysis could be generated with all the modelers experts of the seminar based on some laboratory and model simulations.

## MATERIAL AND METHODS

### Chemical sequential extractions

The new characterization methodology was detailed on (Jimenez *et al.*, 2014, 2015a). After drying and grinding (1mm) the sample, sequential extractions (30 mL) are performed on 0.5g of the dried sample. The fractionation is illustrated by the Figure1. The obtained fractions are:

- Soluble Extractible Organic Matter (SPOM) obtained by washing 4 times 0.5 g of the sample with 30 mL of milli-Q water solution containing 10mM of CaCl<sub>2</sub> during 15 min at 30°C and 300 rpm,
- Readily Extractible Organic Matter (REOM) obtained by shaking the remaining pellet 4 times with saline basic extraction (30 mL of NaCl and NaOH 10 mM) during 15min, at 30°C and 300 rpm,
- Slowly Extractible Organic Matter (SEOM) obtained by 4 sequential strong basic extractions (30 mL of NaOH 0.1 M) of the remaining pellet during 4 h, at 30°C and 300 rpm
- Poorly Extractible Organic Matter (PEOM) obtained by 2 sequential acid extractions (25 mL H<sub>2</sub>SO<sub>4</sub>, 72%) of the remaining pellet during 3 h, at 30°C and 300 rpm.
- The Non Extracted Organic Matter (NEOM) fraction is calculated by subtracting the total organic matter extracted from the sample initial organic matter.



**Figure 1.** Accessibility characterization by sequential extractions protocol

The chemical oxygen demand (COD) of sludge and extracts was measured according to ISO 15705:2002 in order to characterize the organic matter content of each fraction. The validity range of the assay is from 22 to 1 500 mg. L<sup>-1</sup> of oxygen. A sample of 2 mL is required. Samples containing high OM concentrations can be previously diluted in milliQ water. For the negative control, 2 mL of milliQ water was added in the test tube. Tubes were then submitted to incubation at 150°C for 2 h and the resulting oxygen consumption was determined by photometry.

Results are expressed in COD ( $\text{gO}_2\cdot\text{gTS}^{-1}$ ). The extracted fractions are analysed by 3D fluorescence spectroscopy highlighting molecules complexity and correlated with their biodegradability (Jimenez *et al.*, 2014; Jimenez *et al.*, 2015b).

### Methane production rate curves

Methane production rate curves were used from several experiments of anaerobic digestion of organic residues (sludge, fruits, vegetables, manure) after successive 8 batch tests (to reach biomass acclimation) in a 8.5L lab scale reactor with a liquid volume of 6L. The biogas was monitored with a flowgas meter Ritter<sup>®</sup>. The organic load was about 1gVS/L for one batch and substrate/inoculum ratios low ( $<0.1 \text{ gVS/gVS}$ ).

For the wheat straw, after each extraction step, the remaining pellet was used as substrate for batch anaerobic digestion test in order to evaluate each fraction's biodegradation, following the biochemical methane potential (BMP) test proposed by Angelidaki & Sanders (2004).

Concerning the "sludge" experiment, the data come from the Jimenez *et al.* (2015b) work. A 5L lab scale reactor was fed by a mixture of mainly thickened secondary sludge (70% of raw mass), crispbread (18% of raw mass) and growing mix (12% of raw mass). The reactor was working as a semi-continuous one. The organic loading rate was 1.5 gCOD/L.d. Production of biogas and methane were recorded during more than 50 days.

### Model implementation

Using the modified Anaerobic Digestion model n°1 (ADM1, Batstone *et al.*, 2002), input variables of ADM1 were replaced by the fractionation simulating accessibility, i.e. SPOM, REOM as readily hydrolyzable fraction  $X_{RC}$ , SEOM as moderately hydrolysable  $X_{MC}$  and PEOM and NEOM as slowly hydrolysable  $X_{SC}$  and the hydrolysis kinetics as Contois (saturation) kinetics model (Jimenez *et al.* 2015b), as equation 1 describes. Indeed, the results obtained by Jimenez *et al.* (2014) on sludge biodegradation showed this type of chemical accessibility.

An "switching" function is introduced in order to simulate the sequential hydrolysis (Equation 2 and 3). The fluorescent percentages of each fraction were used to predict biodegradability of each fraction during anaerobic digestion.

$$\rho(i) = k_m \times \frac{S^{(i)}/X}{K_{S(i)} + S^{(i)}/X} \times X \times F_{accessibility} \quad (i) \quad \text{Equation 1}$$

If  $i = 1$ ,  $S = X_{rc}$  and  $F_{accessibility} = 1$

$$\text{If } i = 2, S = X_{mc} \text{ and } F_{accessibility} = \frac{1}{1 + X_{rc}/K_I X_{rc}} \quad \text{Equation 2}$$

$$\text{If } i = 3, S = X_{sc} \text{ and } F_{accessibility} = \frac{1}{1 + X_{mc}/K_I X_{mc}} \quad \text{Equation 3}$$

With

$k_m$  is the growth rate of hydrolytic bacteria ( $\text{d}^{-1}$ )

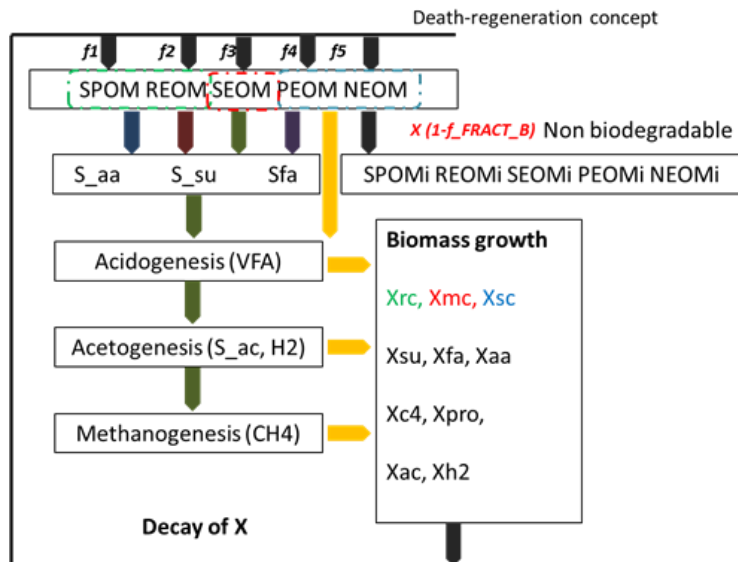
$K_S$  is the half-saturation constant of hydrolytic bacteria ( $\text{d}^{-1}$ )

$S$  is the amount of organic matter contained in the fraction considered ( $\text{kg COD/m}^3$ )

$X$  is the hydrolytic biomass ( $\text{kg COD/m}^3$ )

$F_{accessibility}$  is a switching function based on accessibility degree of the substrate (-)

$K_I$  is the switching concentration for a fraction to another in the switching function ( $\text{kg COD/m}^3$ ).



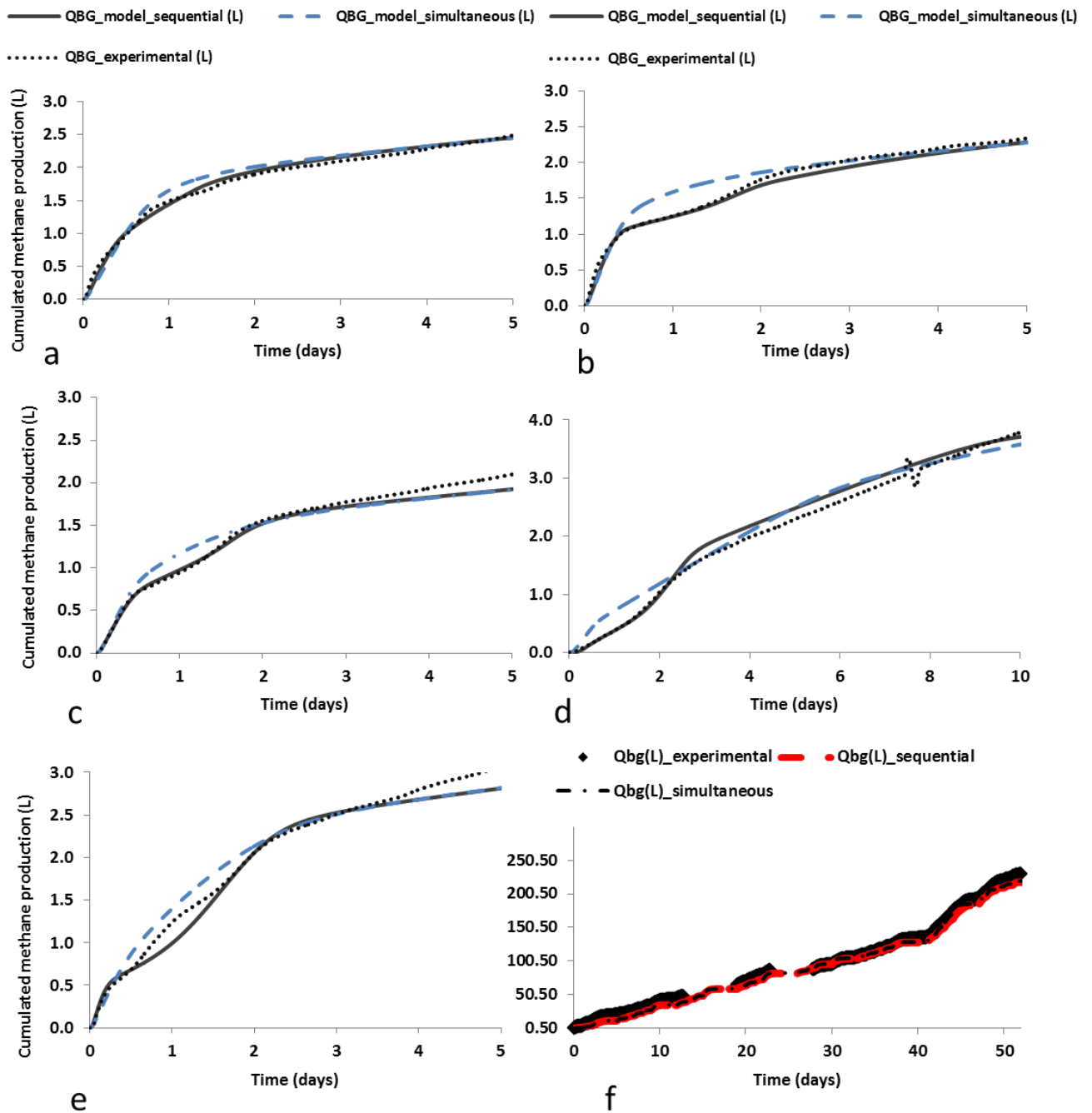
**Figure 2.** Schematic overview of the modified anaerobic digestion model based on ADM1 (Jimenez et al., 2015b)

## RESULTS AND DISCUSSION

### Results obtained on several substrates

Several substrates were tested on successive batches. Results of biogas production resulting from these batch tests are summarised in the Figure 3. Simulations obtained with the simultaneous (i.e. no accessibility inhibition in the model) and sequential models (i.e. accessibility inhibition) are presented also in the Figure 3. In many cases, the modifications of the kinetics with the sequential concept had no impact, as for carrots (Figure 3a) or the secondary sludge (Figure 3f). However, some of them were better modeled with sequential model than with the simultaneous one. This the case for cauliflower, lettuce and wheat straw (respectively Figures 3b, 3c, 3d). Consequently, the use of sequential concept for all the substrates would be a solution in order to reach a good fit of all the MPR curves for all substrates, above all when the rate increases.

However, neither sequential model nor simultaneous model fitted experimental data of the potato biodegradation (Figure 3e). In this case, the hypothesis proposed to explain that is the number of biodegradable fractions (3 fractions in these models) seemed not be sufficient.



**Figure 3.** Methane Production Rate curves obtained experimentally and by simulations with simultaneous model and with sequential model (a: carot, b: cauliflower, d: lettuce, d: wheat straw , e: potato, f: wastewater treatment sludge from Jimenez *et al.* (2015b))

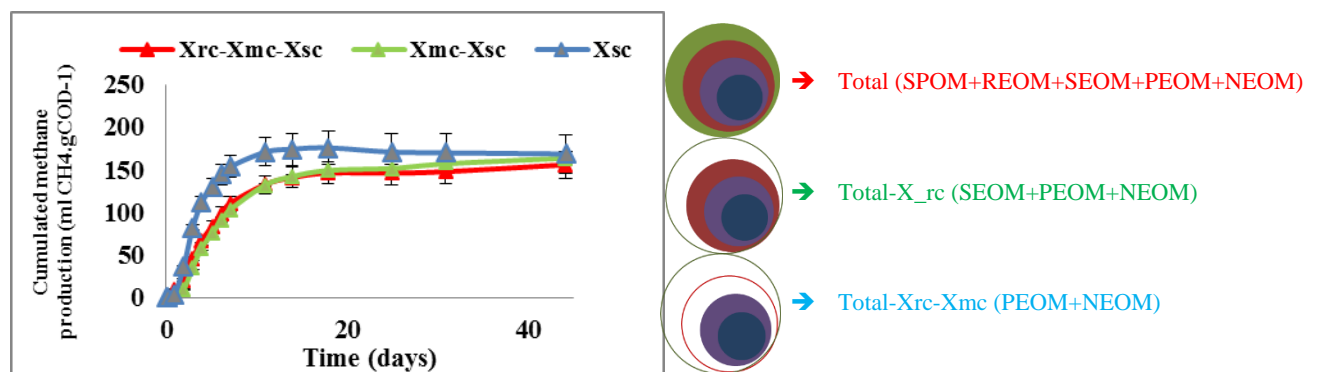
**Case of wheat straw: model substrate for lignocellulosic substrates**

In order to progress, the remaining pellets from wheat straw fractionation were incubated in BMP tests. Three cumulated biogas production curves were obtained from: total substrate, total substrate after SPOM and REOM extractions, and total substrate after SPOM, REOM and SEOM extractions. Results obtained are presented in the Figure 4.

In the case of wheat straw,  $X_{RC}$  fraction was not very important: hence the similarity between total substrate and the total substrate minus  $X_{RC}$  biodegradability curves. However, when the BMP test was performed on the  $X_{SC}$  fraction only, the rate increased, compared with the total substrate-  $X_{RC}$ , and the specific methane production were the same for the 3 experiments.

The extraction step to remove the SEOM fraction acts like an alkaline pre-treatment, allowing the solubilization of some part of recalcitrant lignin-like protecting the wheat straw. Indeed, generally vegetals are composed of lignin wall which protect the plant. In the wheat straw case, a wax layer protects another layer containing cellulose and pectins (pectin is water soluble). SEOM extraction removed the wax layer and allow a quicker biodegradation of PEOM and NEOM fractions (i.e. hemicellulose and cellulose).

This means that the non-accessible feature of  $X_{SC}$  limits hydrolysis although the high biodegradable potential of  $X_{SC}$ . Similar results were obtained by (Rincker *et al.* 2013) after pre-treatments applied on lignocellulose-like substrates. According to the authors, the lag phase could correspond to a colonization process. This colonization phase was also observed in the case of cellulosic fibers with low lignin content (toilet paper) found in primary sludge (Ginestet *et al.*, 2001).

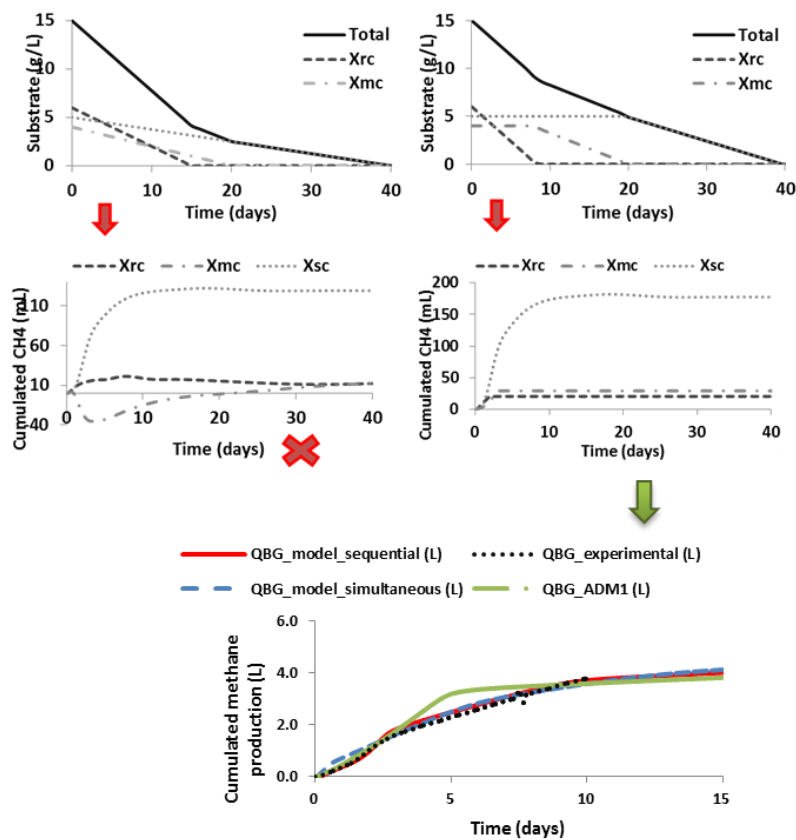


**Figure 4.** Wheat straw anaerobic biodegradation

Moreover, if we want to recover each fraction biodegradation kinetic from these results, the Figure 5 shows that  $X_{SC}$  fraction kinetic is negative. This result is only true if we consider that all the hydrolysable fractions are hydrolysed in a simultaneous way (scenario 1). If we want to recover a positive  $X_{MC}$  biodegradation kinetic, another scenario would be to consider that the fraction  $n$  is not hydrolysed until the fraction  $n-1$  reaches a minimal concentration. That corresponds to the scenario 2 proposed by the Figure 5. In this way, the switching function proposed has to be used in the hydrolysis modeling of each fraction.

The methane production rate curve has a “plateau” in the second part of degradation kinetic. Yasui *et al.* (2008) observed also time lag phenomenon and this kind of “plateau” between 2 fractions of organic matter from several primary sludge. As no inhibition phenomenon was noticed, the authors proposed a sequential model of hydrolysis based on the particle size to explain this observation. This new concept has been successfully applied on sludge anaerobic digestion modeling with a fed-batch lab scale reactor (fed by sludge) in order to predict the methane production rate curves.

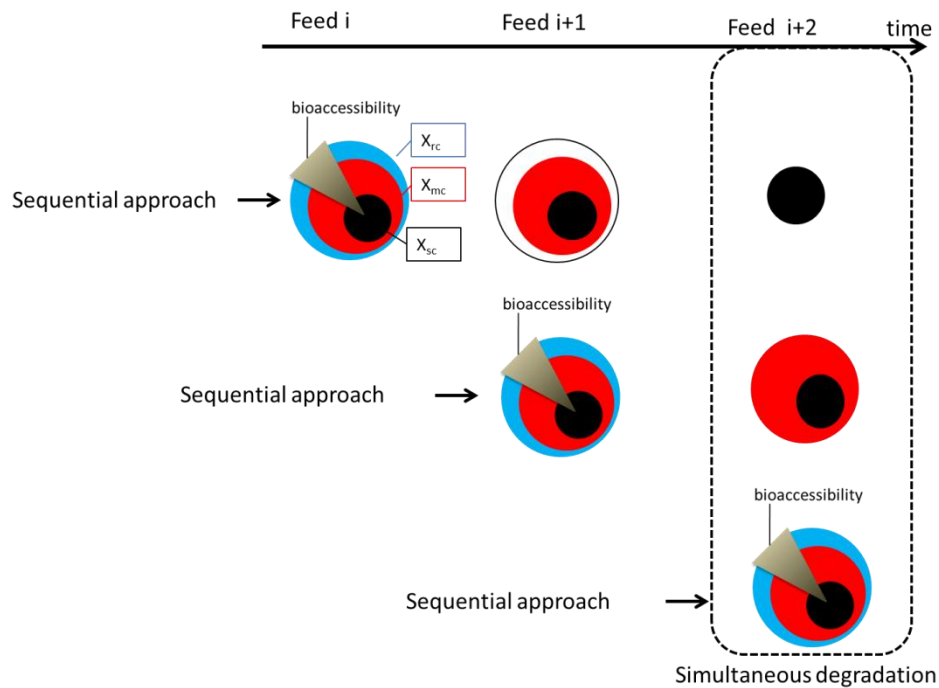




**Figure 5.** Conceptual scenarios proposed to explain accessible fractions hydrolysis kinetics and fed-batch digestion of wheat modelling

Up to now, we have used only batch data (or fed-batch with no biodegradable substrate accumulation between 2 feeds) in order to calibrate the new model. However, the question of its identifiability in the continuous or co-digestion cases could be difficult. Indeed, if we consider that sequential approach governs hydrolysis mechanisms, the hydrolysis mechanisms tend to a simultaneous biodegradation approach, after  $i$  substrate's feeding added in the bioreactor, as illustrated by the Figure 6.

Consequently, the challenge will be to find a way to identify kinetics parameters. Using batch tests could be a solution, but will be the parameter values obtained in batch reactors be transposable to continuous reactors?



**Figure 6.** Illustration of the sequential approach in a continuous reactor

## CONCLUSIONS

The challenge of the paper was to induce discussion about the hydrolysis modelling concepts, i.e. simultaneous versus sequential approaches. To illustrate the discussion and challenge the simultaneous approach, some case studies obtained experimentally were modelled to test both concepts. Also, a new fractionation methodology was introduced in order to deeply study the hydrolysis mechanisms. This methodology, based on accessibility characterization, was successfully used to replace the ADM1 variables describing the particular organic matter. The methodology allowed also to test both hypothesis about simultaneous versus sequential hydrolysis. The sequential approach fitted all the substrates biodegradation while simultaneous approach was sometimes not applicable (like for wheat straw or lettuce biodegradation). Although this model is working, it represents an hypothesis considering hydrolysis mechanisms. The demonstration has to be determined. A proof of the concept has to be defined and an experimental set-up should be designed, with a specific accessibility fractions study.

Finally the question of how these mechanisms (successive degradation, colonization) should be taken into account for modelling continuous systems will be debated. Solid particles have a distribution of retention time in such systems, with different degree of degradation/colonization. The population mass balance models seem appropriate to describe this heterogeneity in hydrolysis (Lebaz *et al.*, 2015) but simplified approaches would be also probably suitable.

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