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Modeling anaerobic digestion of microalgae using ADM1

Francis Mairet [a,b,*], Olivier Bernard [a], Monique Ras [c], Laurent Lardon [c,a], Jean-Philippe Steyer [c,a]

[a] BIOCORE-INRIA, BP93, 06902 Sophia-Antipolis Cedex, France
[b] Departamento de Matemática, Universidad Técnica Federico Santa María, Avda España 1680, Valparaíso, Chile
[c] INRA, UR050, Laboratoire de Biotechnologie de l'Environnement, Avenue des Etangs, Narbonne F-11100, France

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ABSTRACT

The coupling between a microalgal pond and an anaerobic digester is a promising alternative for sustainable energy production by transforming carbon dioxide into methane using solar energy. In this paper, we demonstrate the ability of the original ADM1 model and a modified version (based on Contois kinetics for the hydrolysis steps) to represent microalgae anaerobic digestion. Simulations were compared to experimental data of an anaerobic digester fed with *Chlorella vulgaris*. The modified ADM1 fits adequately the data for the considered 140 day experiment encompassing a variety of influent load and flow rates. It turns out to be a reliable predictive tool for optimising the coupling of microalgae with anaerobic digestion processes.

1. Introduction

Microalgae hold a significant promise as a sustainable supplier of raw materials for the food and chemical industries, as well as for CO_2 mitigation and biofuel production (Spolaore et al., 2006; Chisti, 2007; Wijffels and Barbosa, 2010). Anaerobic digestion can be applied to convert microalgae biomass to biogas (Sialve et al., 2009; Zamalloa et al., 2011) whether using the total produced biomass or the residual fraction remaining after extraction of valuable products (*e.g.* triglycerides or carotenoids). This process not only recovers the energy stored in biomass, but also leads to ammonium and phosphate release, which can in turn be source of nutrients for the microalgae culture. Coupling microalgae culture and anaerobic digestion is therefore a promising process to convert solar energy into methane. Nevertheless, anaerobic digestion of microalgae faces several hurdles (Sialve et al., 2009; Mussgnug et al., 2010):

- For some species, refractory compounds found in the cell wall can lead to a low biodegradability.
- High ammonia concentration resulting from the degradation of the high nitrogen content of microalgae, can inhibit bacterial growth, especially methanogenic bacteria (Koster and Lettinga, 1984; Chen et al., 2008).
- In the case of marine microalgae, plausible toxic effects can reduce the digester performance.

A dynamical model of microalgae anaerobic digestion can therefore help apprehend the complexity of the process and also identify optimal working strategies. Moreover, the coupling between a microalgal production unit and a digester raises particular design and control issues which are difficult to solve without a simulation model.

Modelling of anaerobic digestion has been widely developed since the seventies (Lyberatos and Skiadas, 1999), from simple models (e.g. considering one limiting reaction (Graef and Andrews, 1974; Donoso-Bravo et al., 2010) or two reactions Bernard et al., 2001) to more realistic representations (e.g. the IWA anaerobic digestion model # 1 – ADM1 – (Batstone et al., 2002) with 19 biochemical reactions).

However, to our knowledge, none of these models has yet been applied using microalgae as feedstock. In this paper, our aim is to investigate the ability of ADM1 to describe microalgae anaerobic digestion.

The article is structured as follows: after a first description of the digestion experiment, ADM1 is presented and some modifications are proposed. The simulation and calibration procedures are described and the model is compared to experimental data provided by anaerobic digestion of the freshwater microalgae *Chlorella vulgaris* (Ras et al., 2011). Finally, simulations are performed in order to evaluate and discuss process performances.

* Corresponding author at: Departamento de Matemática, Universidad Técnica Federico, Santa María, Avda España 1680, Valparaíso, Chile. Tel.: +56 32 265 47 85; fax: +56 32 265 45 63.
E-mail address: francis.mairet@usm.cl (F. Mairet).

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2. Methods

2.1. Digestion experiment

2.1.1. Digester set-up

Anaerobic digestion of microalgae was performed over 140 days in a continuously mixed reactor at 35°C without pH control. The reactor was fed daily with a concentrated stock of *C. vulgaris* harvested by settling from a photobioreactor production unit. The amount of organic biomass introduced in the digester per day was fixed by the harvesting rate and was maintained constant at 1 gCOD L^{-1} d^{-1}. In order to undergo constant and controlled hydraulic retention times (HRT) over long periods, the concentration of the influent was standardised with demineralised water. For each addition, the same liquid volume was withdrawn in order to maintain a constant reactor liquid volume. Fig. 1 shows the daily dilution rate average together with the substrate additions. At the end of the experiment (from day 100 to 120), the microalgae substrate loaded in the digester was increased by successive inputs of 1, 2, 4 and 6 gCOD in order to provide a further insight into the dynamics of microalgae degradation.

2.1.2. Measurements

The following measurements were performed: biogas volume (by water displacement), biogas composition (by gas chromatography), VFA concentrations (by gas chromatography), ionic concentrations (by ion chromatography), pH and chemical oxygen demand (by colorimetric method).

Further details on the experimental protocols and material and methods can be found in Ras et al. (2011).

2.2. Modeling approach

2.2.1. ADM1

ADM1 (Batstone et al., 2002) describes the different steps of anaerobic digestion: disintegration, hydrolysis, acidogenesis, acetogenesis, and methanogenesis. This model accounts for 19 biochemical reactions associated to 7 bacterial populations. Biomass growth is considered as proportional to substrate uptake. The kinetics are described according to a Monod function of the substrate, associated with pH, hydrogen and ammonia inhibition terms. ADM1 also includes the physico-chemical reactions: liquid–gas transfer, acid–base reactions and pH computation. This model has been widely used to describe the anaerobic digestion of various substrates (Batstone et al., 2006; Parker, 2005).

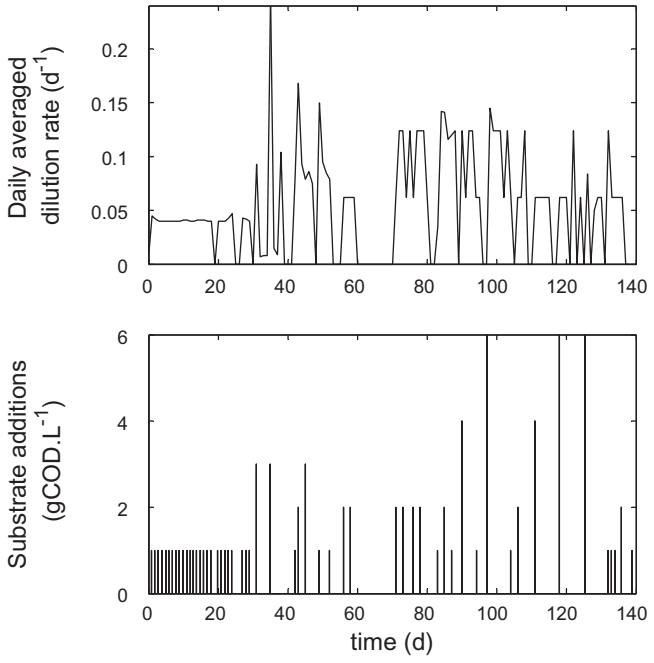

Fig. 1. Operating conditions for the anaerobic digestion of *Chlorella vulgaris*.

2.2.2. Modification of the hydrolysis step

Hydrolysis is a complex multi-step process which is not well understood. In ADM1, the hydrolysis rates are taken as first order kinetics. This expression has appeared to be a simple and reliable way of representing the reaction. In some cases, hydrolysis can be better represented by the Contois model (Vavilin et al., 2008), which assumes that the kinetics do not depend on the substrate concentration, but on the amount of substrate per biomass unit. Thereby, Ramirez et al. (2009) have proposed a modified ADM1 version using Contois model associated to the growth of hydrolytic bacteria.

Hydrolysis is generally admitted as one of the limiting steps of microalgae digestion. This reaction must hence be considered with particular attention. In this study, we consider here that the enzymes are produced by the bacterial population which consumes the outcoming products of hydrolysis. We therefore suggest to use the Contois model associated to the benefiting bacteria population. For example, the hydrolysis of carbohydrate X_{ch} produces sugar S_{su} which are consumed by the bacteria population X_{su}. The hydrolysis rate of carbohydrate becomes a Contois function of substrate X_{ch} and biomass X_{su}. The modifications of the hydrolysis rates are presented in Table 1.

The authors wish to underline that this choice does not require new bacterial populations but only a modification of the reaction rates, so implementation in any existing ADM1 simulator is straightforward.

2.2.3. ADM1 implementation

ADM1 was simulated using the Rosén and Jeppsson (2006) implementation. Reaction rates ρ_2, ρ_3, and ρ_4 are taken according to Table 1. Moreover, feeding with impulses generates transients in the transfer from the gas to the liquid (*i.e.* a negative specific mass transfer rate of CO_2 : $\rho_{T,10} < 0$), with pressure in the headspace P_{gas} which can become smaller than P_{atm}. A modification of the physico-chemical reaction is therefore necessary when modelling the liquid–gas transfer. Computing the gas flow rate as the sum of the liquid-gas transfer rates (as proposed in Batstone et al. (2002)) is therefore incorrect. The alternative solution consists in computing the gas flow rate from an overpressure in the headspace:

$$q_{gas} = k_p(P_{gas} - P_{atm})\frac{P_{gas}}{P_{atm}} \quad (1)$$

with k_p the pipe resistance coefficient (Batstone et al., 2002).

This solution is chosen with a slight modification to consider the case $P_{gas} < P_{atm}$:

$$q_{gas} = max\left(0; k_p(P_{gas} - P_{atm})\frac{P_{gas}}{P_{atm}}\right) \quad (2)$$

Table 1
Hydrolysis rates.

Substrate	Rate	Original ADM1	Modified ADM1
Carbohydrate	ρ_2	$= k_{hyd,ch}X_{ch}$	$= k^*_{hyd,ch}\frac{X_{ch}}{K_{S,ch}X_{su}+X_{ch}}X_{su}$
Protein	ρ_3	$= k_{hyd,pr}X_{pr}$	$= k^*_{hyd,pr}\frac{X_{pr}}{K_{S,pr}X_{aa}+X_{pr}}X_{aa}$
Lipid	ρ_4	$= k_{hyd,li}X_{li}$	$= k^*_{hyd,li}\frac{X_{li}}{K_{S,li}X_{fa}+X_{li}}X_{fa}$

2.2.4. Experiment simulation

The experiment was carried out with feeding impulses. In order to avoid numerical error due to the impulses, simulations were reinitialised after each substrate addition, so that the dynamics between two pulses is perfectly continuous. The effect of each addition (at time t_i) on the concentrations (gathered in vector ξ) are computed from a mass balance as follows:

$$\xi(t_i^+) = \xi(t_i^-) + \frac{V_{in}(t_i)}{V_{liq}}\left(\xi_{in}(t_i) - \xi(t_i^-)\right) \quad (3)$$

where V_{in} and ξ_{in} are the volume and the concentrations of the feed additions.

2.2.5. Influent characterisation

The input characterisation is a critical step in modelling anaerobic digestion (Kleerebezem and Van Loosdrecht, 2006). The inlet concentration was 30 kgCOD m^{-3} with approximately 90% of particulate matter. We assume that the soluble COD is mainly composed of sugars (Hulatt and Thomas, 2010). This leads to $X_{c,in} = 27$ kgCOD m^{-3} and $S_{su,in} = 3$ kgCOD m^{-3}. pH in the influent was not monitored but it ranges between 9 and 10 (this high pH results from CO_2 uptake by microalgae in the settler). Inorganic carbon in the influent is computed assuming CO_2 at equilibrium with its atmospheric partial pressure. Then, pH is computed on the basis of CO_2 ($= K_{H,CO_2} P^{atm}_{CO_2}$), $S_{cat,in}$ and $S_{an,in}$ which drive the charge balance. The input characterisation results are given in Table 2.

2.2.6. Parameter identification

Coefficients $f_{ch,xc}, f_{pr,xc}, f_{li,xc}, f_{xI,xc}$, and $f_{sI,xc}$ represent the fraction of the substrate into the different intermediates, they hence have to be identified according to the substrate composition. The microalgae composition is species dependent but it can also vary with environmental conditions (Harrison et al., 1990; Mairet et al., 2011). The average biochemical composition for *C. vulgaris* in non-limited growth conditions is given in Table 3 (Becker, 2007; Pruvost et al., 2011). Using approximate elemental compositions (see Table 3) proposed by Geider and Roche (2002), this biochemical composition leads to a C/N ratio of 5.9, which is in line with the measured ratio of 6. The conversion from dry matter (DM) *COD* is computed using the approximate elemental compositions. The inert fraction is computed from the experimental data of batch experiments (data not shown). Assuming that the inert fraction is only particulate (soluble COD stayed low during the experiment) with the same composition than the algae, we can finally compute all the parameters $f_{.,xc}$ (Table 4). Nitrogen content of composites N_{xc} and inert N_I is also deduced from the average biochemical composition of *C. vulgaris*.

The pH inhibition terms turn out to strongly affect the methanogenesis step. Since such inhibition was not observed experimentally, a lower value of the $pH_{LL,ac}$ parameter is used (5.2 instead of 6).

Regarding the original ADM1, the other parameter values were not modified. Parameter values of the Contois model are identified using a minimisation procedure (function fminsearch under Matlab®). This algorithm, based on the Simplex search method, is used to find the set of parameters that minimises a square-error criterion between the model and the measurements. Parameter values modified from the original ADM1 are given in Table 4.

Table 2
Input characterisation.[a]

Parameter	Value	Meaning
$S_{su,in}$	3 kgCOD m^{-3}	Sugar concentration
$X_{c,in}$	27 kgCOD m^{-3}	Composite concentration
$S_{IC,in}$	0.019 M	Inorganic carbon concentration
$S_{IN,in}$	0.011 M	Inorganic nitrogen concentration
$S_{cat,in}$	0.024 M	Inert cation concentration
$S_{an,in}$	0.0065 M	Inert anion concentration
pH_{in}	9.6	

[a] The other state variables are null.

Table 4
Parameter values modified from ADM1.

Parameter	Value	Meaning
Stochiometric parameters[a]		
$f_{sI,xc}$	0 (0)	Yield of soluble inert on composites
$f_{xI,xc}$	0.3 (0.3)	Yield of particulate inert on composites
$f_{ch,xc}$	0.08 (0.2)	Yield of carbohydrates on composites
$f_{pr,xc}$	0.40 (0.15)	Yield of proteins on composites
$f_{li,xc}$	0.22 (0.45)	Yield of lipids on composites
N_{xc}	0.0037 (0.0011) kmol kgCOD^{-1}	Nitrogen content of composites
N_I	0.0037 (0.0011) kmol kgCOD^{-1}	Nitrogen content of inert
Kinetic parameters		
$pH_{LL,ac}$	5.2	pH inhibition coefficient
$k^*_{hyd,ch}$	3.18 day^{-1}	Maximum specific hydrolysis rate of carbohydrates
$K_{S,ch}$	0.50 kg COD m^{-3}	Contois half saturation constant of carbohydrate hydrolysis
$k^*_{hyd,pr}$	1.04 day^{-1}	Maximum specific hydrolysis rate of proteins
$K_{S,pr}$	0.26 kgCOD m^{-3}	Contois half saturation constant of protein hydrolysis
$k^*_{hyd,li}$	3.07 d^{-1}	Maximum specific hydrolysis rate of lipids
$K_{S,li}$	0.49 kgCOD m^{-3}	Contois half saturation constant of lipid hydrolysis

[a] The first value stands for non-limited microalgae, the bracketed one for nitrogen-starved microalgae.

3. Results and discussion

3.1. Comparison with experimental data

3.1.1. Original ADM1

The original version of ADM1 shows a good ability in describing microalgae digestion (see Figs. 2–4), except at the end of the experiment when a high dilution rate was applied and where the model

Table 3
Microalgae composition.

	DM basis (%)			COD basis (%)			COD content (kgCOD kgDM^{-1})	N content (kmol N kgCOD^{-1})
	Pr	Li	Ch	Pr	Li	Ch		
Protein $C_{4.43}H_7O_{1.44}N_{1.16}$	100	0	0	100	0	0	1.76	0.0065
Lipid $C_{40}H_{74}O_5$	0	100	0	0	100	0	2.83	0
Carbohydrate $C_6H_{12}O_6$	0	0	100	0	0	100	1.07	0
Non-limited microalgae	60	20	20	57	31	12	1.84	0.0037
N-starved microalgae	20	50	30	17	68	15	2.09	0.0011

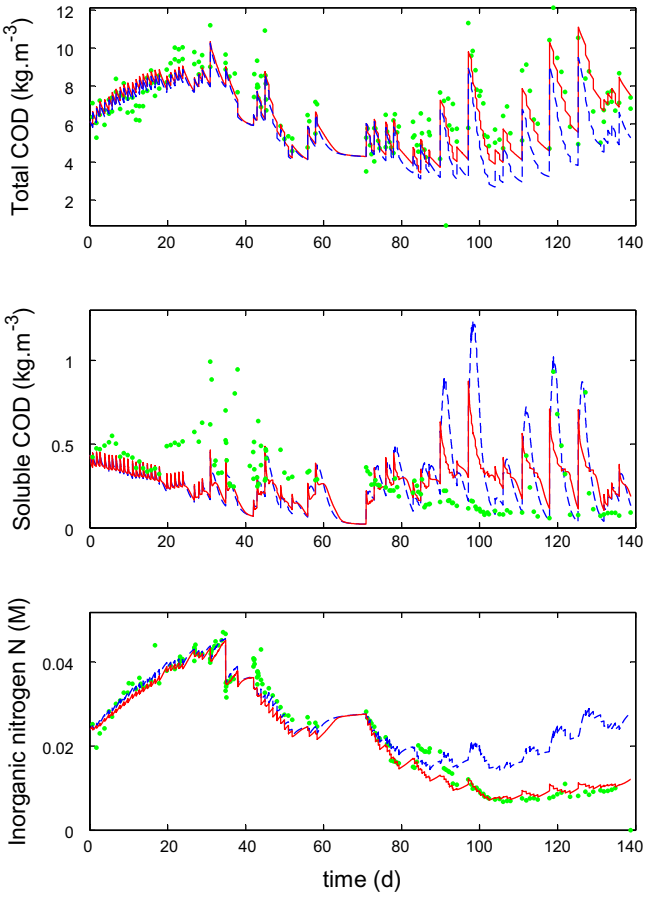

Fig. 2. Total COD, soluble COD and inorganic nitrogen concentrations: comparison between the original ADM1 (blue dashed lines), the modified ADM1 (red lines) and experimental data (green dots) of *Chlorella vulgaris* digestion. (For interpretation of the references to colour in this figure legend, the reader is referred to the web version of this article.)

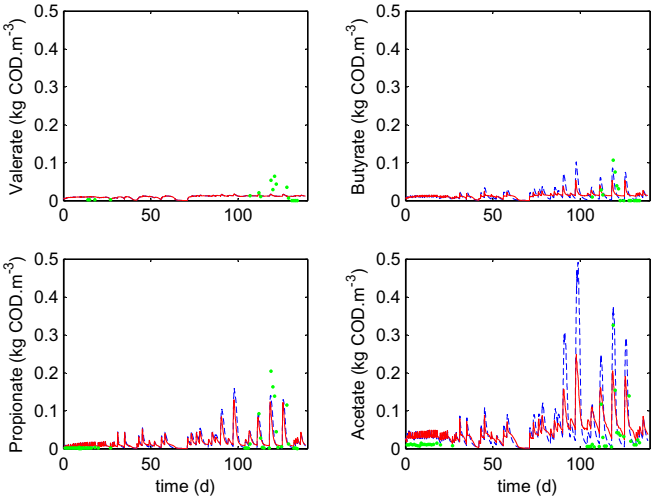

Fig. 3. VFA concentrations: comparison between the original ADM1 (blue dashed lines), the modified ADM1 (red lines) and experimental data (green dots) of *Chlorella vulgaris* digestion. (For interpretation of the references to colour in this figure legend, the reader is referred to the web version of this article.)

overestimates inorganic nitrogen release. The low experimental ammonium release means that there is an accumulation of nitrogen compounds, such as proteins X_{pr} or amino-acids S_{aa}. As the soluble COD remains low, we can assume that there is an

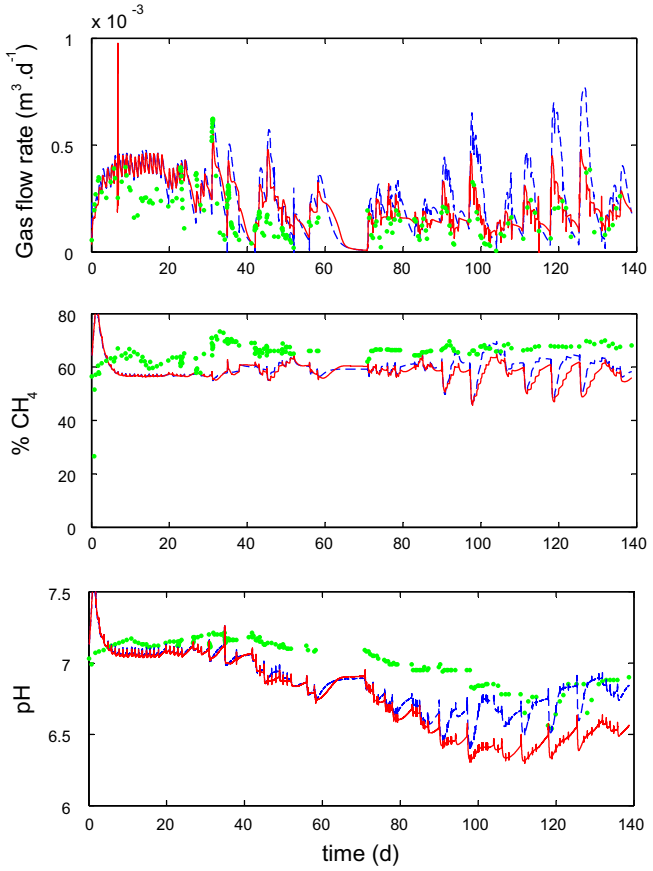

Fig. 4. Gas flow rate, gas composition and pH: comparison between the original ADM1 (blue dashed lines), the modified ADM1 (red lines) and experimental data (green dots) of *Chlorella vulgaris* digestion. (For interpretation of the references to colour in this figure legend, the reader is referred to the web version of this article.)

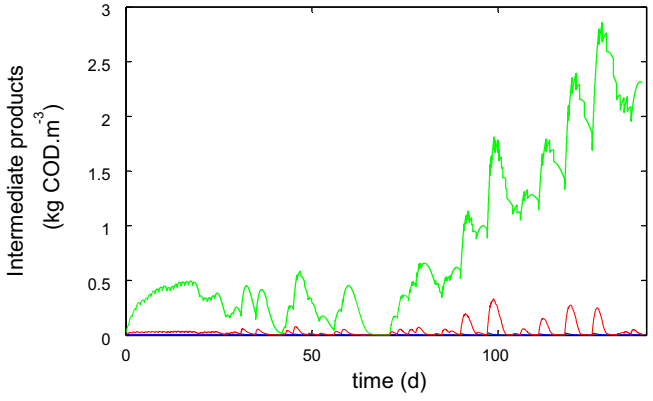

Fig. 5. Model prediction of carbohydrate (blue line), protein (green line) and lipid (red line) concentrations during *Chlorella vulgaris* digestion. The high dilution rate at the end of the experiment leads to an accumulation of proteins. (For interpretation of the references to colour in this figure legend, the reader is referred to the web version of this article.)

accumulation of X_{pr} together with a low protein hydrolysis rate ρ_3. The original ADM1 could not reproduce these dynamics even after adapting the parameter values.

3.1.2. Modified ADM1

The modified ADM1 describes accurately the experimental data. In particular, the good representation of inorganic nitrogen concentrations (Fig. 2) is a first hint that using the Contois model for

the hydrolysis step effectively improves ADM1 ability to describe microalgae digestion.

The model predicts low VFA concentrations (Fig. 3), except during transients after the successive increasing inputs at the end of the experiment (after day 100), which is in agreement with the experimental data. The gas flow rate is well predicted (Fig. 4), but the methane content is slightly underestimated. This discrepancy for methane content is probably due to pH underestimation. A better characterisation of the input (mainly the inorganic carbon concentration $S_{IC,in}$, which can be computed from input pH measurements) should improve the predictions of pH and methane content. Note that $S_{IC,in}$ probably did vary during the 140 day experiment. Since the input pH has not been measured, $S_{IC,in}$ was estimated assuming an equilibrium between dissolved CO_2 and its atmospheric partial pressure (thus limiting the number of identified parameters). The feeding impulses generates peaks in the pH which can be observed both in the simulations and in the experimental data (at days 112, 119 and 128). These impulses also lead to peaks in the simulated methane content, while no significant change was observed experimentally. This discrepancy suggests that the simple liquid-gas transfer model in ADM1 is not suitable to represent such strong disturbances.

Since all the intermediate substrates or products were not measured separately, estimations of their dynamics can be obtained with model simulation (Fig. 5). From the 50th day onwards, when a high dilution rate was applied, the modified ADM1 predicts an accumulation of protein X_{pr}. On the other hand, carbohydrates and lipids are almost completely hydrolysed because of a higher maximal hydrolysis rates of X_{ch} and X_{li}. The above phenomena lead to a release of inorganic nitrogen which was not correlated to the methane production, as it was observed experimentally (Ras et al., 2011).

3.1.3. pH inhibition

In ADM1, pH inhibition is included in the kinetics of all the microbial populations, using the following function:

$$I_{pH,xx} = \begin{cases} \exp\left(-3\left(\frac{pH-pH_{UL,xx}}{pH_{UL,xx}-pH_{LL,xx}}\right)^2\right) & : pH < pH_{UL,xx} \\ 1 & : pH > pH_{UL,xx} \end{cases} \quad (4)$$

where $pH_{UL,xx}$ and $pH_{LL,xx}$ are pH values at which no inhibition takes place and at which the group of organisms are 95% inhibited respectively. For the considered experimental pH range (*i.e.* from pH 6 to pH 7), the model predicts that only the acetate-utilising methanogens are inhibited. Indeed, with the original ADM1 value, the simulation shows reactor acidification: when the pH reaches 6.5, methanogens are strongly inhibited and the pH abruptly drops due to VFA accumulation. In the experiment, the pH drops to 6.5 right after feeding and then comes back to its equilibrium value. This clearly shows that the methanogens were less sensitive to pH than the initial model kinetics. Therefore, parameter $pH_{LL,ac}$

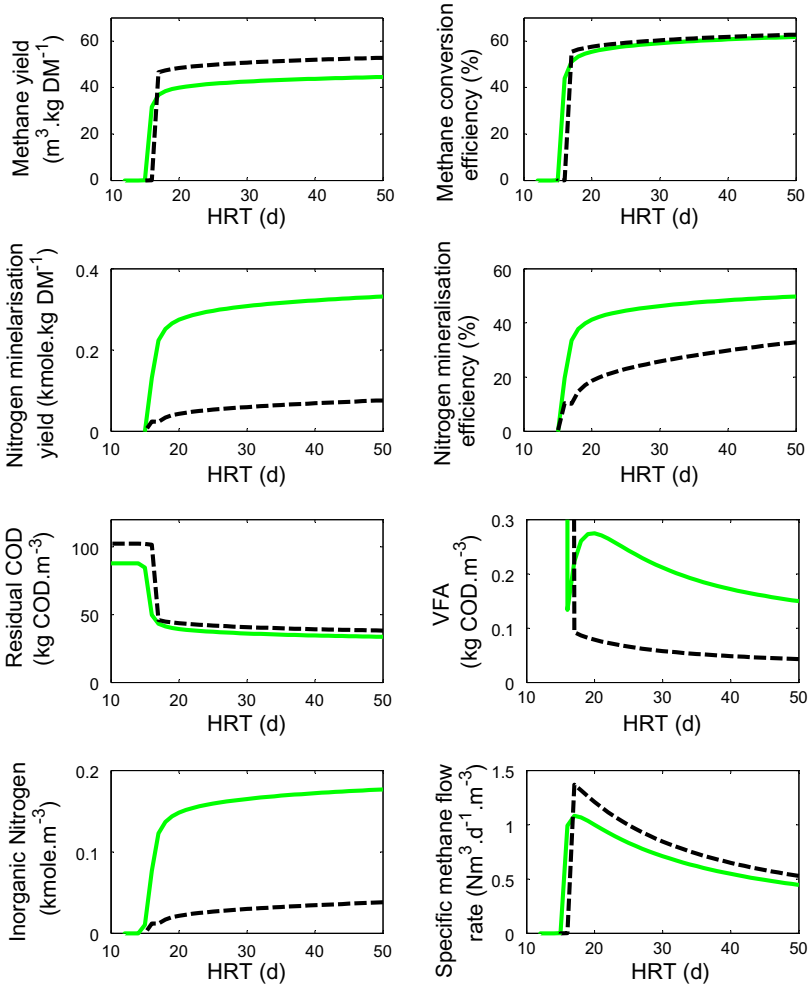

Fig. 6. Performance evaluation of microalgae digestion using ADM1 at steady state. Comparison between non-limited (green solid line) and N-starved (black dashed line) microalgae. (For interpretation of the references to colour in this figure legend, the reader is referred to the web version of this article.)

has been lowered down to 5.2 in order to reduce the inhibition effect.

3.1.4. Parameter values

Including the Contois model for the hydrolysis step in ADM1 improves its ability to describe microalgae digestion. Nevertheless, it adds new parameters which have to be identified. A sensibility analysis (not shown) indicates that with our experimental dataset, the estimation of protein hydrolysis parameters is accurate, but carbohydrate and lipid parameters are questionable. Indeed, in opposition to protein degradation which can be indirectly monitored though inorganic nitrogen concentrations, the distinction between carbohydrates and lipids degradation is tricky. Moreover, the low lipid and carbohydrate contents gives them less influence on the output.

However, values presented in this study (Table 4) can be compared to the literature. The Contois model has been used to represent the anaerobic digestion of brewery-spent grains and household solid waste (assuming that hydrolysis was the limiting step) (Vavilin et al., 2008). The maximum specific uptake rates ($1.5\ day^{-1}$ for brewery-spent grains, $1.25\ day^{-1}$ for waste and $2.5\ day^{-1}$ for residual organic material in inoculum) are consistent with our values. On the other hand, Ramirez et al. (2009) have used higher values ($10\ day^{-1}$ for lipids, proteins and carbohydrates) for representing the hydrolysis with the Contois model in ADM1. Nevertheless, in this study, the disintegration rate is also represented by the Contois model, with a maximum specific rate lower than for hydrolysis ($1.75\ day^{-1}$). Therefore, the hydrolysis rates have probably no influence on the simulation results. In the absence of intermediate product measurements, the distinction between disintegration and hydrolysis is delicate and we can notice that their disintegration rate is close to our hydrolysis rates.

To conclude, the differences between our values and the literature are relatively small in comparison with those observed for the kinetic coefficient of the first-order rate of hydrolysis (Vavilin et al., 2008). This shows that Contois model is more robust to describe a broad range of experiments, while the first-order kinetic is more experiment dependent.

3.2. Estimation of process performances

Although coupling microalgal pond and anaerobic digestion sounds promising, it is crucial to assess its economic and environmental interests. Nevertheless, such studies (as proposed by Collet et al. (2011) and Zamalloa et al. (2011)) need to extrapolate the few lab results to larger scale production, which can be quite tricky. A dynamical model can support these extrapolation studies and is particularly appropriate for such a complex process.

We have used the modified ADM1 to simulate the process in various operating conditions. The inlet concentration is mainly imposed by the microalgae culture and the dewatering step, therefore we focus on the effect of the hydraulic retention time (HRT) on the digester. We use an inlet concentration of $50\ kgDM\ m^{-3}$, which represents a microalgae culture of $0.5\ kgDM\ m^{-3}$ concentrated 100 times (Collet et al., 2011; Zamalloa et al., 2011).

3.2.1. Digester performance

Fig. 6 (solid lines) represents the influence at steady state of the HRT on the digester performance. For HRT lower than 15 days, we observe reactor acidification. In the following, we will focus only on the normal operation (HRT $>$ 15 days). As expected, increasing the HRT leads to a higher methane conversion efficiency until it reaches a plateau (65%, due to the non-biodegradable fraction of microalgae). Nevertheless, in practice, operating the process at a high HRT means an increase in the digester volume to process the same algal inflow and consequently an increase in the energy required to mix and keep the heat of the corresponding volume. The optimal methane production is obtained for a trade-off between the loading rate and the methane conversion efficiency. The nitrogen mineralisation efficiency is of particular interest. Indeed, the ammonium produced during the digestion will be used as a source of nitrogen for the microalgae culture. Therefore a high nitrogen mineralisation efficiency is also expected in order to reduce the environmental impacts of the process. However, this efficiency does not exceed 55% since nitrogen contained in the non-biodegradable fraction of microalgae is not recovered. An additional nitrogen source is hence required when coupling microalgae culture–anaerobic digestion to offset losses.

Based on an extrapolation of (Ras et al. (2011)) experimental results, Collet et al. (2011) have suggested to use a high HRT (46 days) assuming that methane conversion and nitrogen mineralisation efficiencies are respectively 56% and 90% in this condition. This methane conversion efficiency is close to our prediction (61%). Regarding nitrogen mineralisation, the model predicts that the process cannot reach this efficiency. Nevertheless, this prevision is highly dependent on the proportion of nitrogen contained in the non-biodegradable fraction of microalgae (which was not measured in Ras et al. (2011)). It seems that using such a high HRT is not relevant as the methane conversion and the nitrogen mineralisation efficiencies rapidly reach their plateau. A HRT around 20 days seems more relevant as it leads to a high productivity without significantly affecting the efficiencies.

3.2.2. Digester stability in response to overloading

The stability of the process has also been evaluated through simulation. Step increases of the input concentration are simulated for various dilution rates (figures not shown). For some (easily degradable) substrate, such a disturbance can produce an accumulation of volatile fatty acids potentially leading to the acidification of the digester. For microalgae, we observe a transient increase of particulate COD (mainly X_{pr}) followed by a process stabilisation. These transient increases show that hydrolysis is the limiting step. Therefore, an increase of the input concentration does not destabilise the process.

3.2.3. Ammonia toxicity

Sialve et al. (2009) have reported that the ammonium release due to the high nitrogen content of microalgae can be detrimental for the digester because of the inhibitory effect of ammonia. The pH, which triggers the dissociation of the inorganic nitrogen between free ammonia and ammonium, plays an important role in the inhibition.

We first studied the potential risk of process destabilisation. In ADM1, ammonia inhibits only the methanogens. An ammonia increase could therefore produce an accumulation of VFA, which could lead to the acidification of the reactor. In our simulations, this phenomena was not observed because of the high alkalinity (due to ammonium) and also because a pH decrease (due to VFA accumulation) leads to a decrease of ammonia concentration, and thus reduces inhibition. This suggests that there might be a complex interaction between inorganic nitrogen and pH, which tends to stabilise the process.

Moreover, from our simulations, it appears that ammonia hardly decreases digester efficiency. Since hydrolysis is the limiting step, a slight inhibition of methanogenesis does not affect the process.

As mentioned by Sialve et al. (2009), some pretreatments (*e.g.* thermal or ultrasonic treatments) can improve digestion kinetics, in particular the hydrolysis step. Therefore, the methanogenesis could become the limiting step. In this case, the inorganic nitrogen would have a more negative effect on the process, as it would slow down the limiting step.

3.2.4. Should we starve microalgae before digestion?

Nitrogen starvation can strongly increase the lipid content of microalgae (Wijffels and Barbosa, 2010). Sialve et al. (2009) have proposed to use nitrogen-starved microalgae, as starvation increases its calorific value and decreases its nitrogen content, which should improve microalgae digestion. This strategy is evaluated through simulations, using the same DM concentration in the input as for non-limited microalgae. A typical composition of nitrogen-starved microalgae is used to estimate the parameters $f_{\cdot,xc}$ (see Tables 3 and 4).

In comparison with non-limited microalgae, we obtain a small increase of productivity and methane yield (Fig. 6) as the COD content is slightly higher, except for short HRT where nitrogen depletion (because of a low degradation of proteins) leads to a washout. The methane conversion efficiency remains the same. The lower nitrogen content improves the process efficiency only by a small fraction, as nitrogen almost does not affect the process. The nitrogen mineralisation efficiency is smaller for N-starved microalgae because a higher proportion of the nitrogen is consumed by the bacterial populations (the same uptake represents a smaller proportion of the total nitrogen for non-limited microalgae).

Therefore, it seems that nitrogen starvation increases only slightly the digester performances. Moreover, nutrient starvation also affects microalgae productivity, thereby jeopardising the overall productivity of the coupled process. Nevertheless, the question remains whether it is worth starving microalgae for a digestion with pretreatment. In such a case, a low nitrogen content could improve significantly process performances (see Section 3.2.3).

On the other hand, Mairet et al. (2011) have shown that nitrogen limitation (obtained in continuous culture imposing a dilution rate smaller than the maximal growth rate) can increase both carbohydrate content and productivity. Besides its smaller COD content, a higher carbohydrate input can be interesting because of its potential to be degraded more rapidly. Nevertheless, as already mentioned, the estimation of carbohydrate hydrolysis rate is not accurate with our experimental dataset. The model is therefore not totally reliable to evaluate the potential gain of such a strategy.

4. Conclusion

In this work, we have proposed a modified version of ADM1 (including Contois model for hydrolysis) for representing anaerobic digestion of microalgae. This model fits very well the data provided by a 140 day experiment of *C. vulgaris* digestion. Numerical simulations have then been used to evaluate performances of microalgae digestion.

The ability of ADM1 (originally proposed for waste activated sludge (WAS) digestion) to represent microalgae digestion confirms the observation of Ras et al. (2011): WAS and microalgae digestions show similar trends. Therefore, microalgae digestion could probably benefit from the improvement obtained with WAS digestion (pre-treatment, reactor design, simulation and control, etc.).

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