Modelling anaerobic digestion acclimatisation to a biodegradable toxicant: application to cyanide

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Abstract The observed acclimatisation to biodegradable toxicants in anaerobic cassava wastewater treatment is explained by modelling anaerobic cyanide degradation. A complete degradation pathway is proposed for cyanide. Cyanide degradation is modelled as enzymatic hydrolysis to formate and ammonia. Ammonia is added to the inorganic nitrogen content of the digester while formate is degraded by the hydrogenotrophic methanogens. Cyanide irreversible enzyme inhibition is modelled as an inhibition factor to acetate uptake processes. Cyanide irreversible toxicity is modelled as a decay factor to the acetate degraders. Cyanide as well as added phosphorus buffer solution were considered in the chemical equilibrium calculations of pH. The observed reversible effect after acclimatisation of sludge is modelled by a population shift between two aceticlastic methanogens that have different tolerance to cyanide toxicity. The proposed pathway is added to the IWA Anaerobic Digestion Model no.1 (ADM1). The ADM1 model with the designed extension is validated by an experiment using three lab-scale upflow anaerobic sludge bed reactors which were exposed to different cyanide loadings.

Keywords ADM1; cyanide; inhibition; toxicity; upflow anaerobic sludge bed

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Introduction

High rate anaerobic reactors can adapt to treat wastewater contaminated with irreversible toxicants. Acclimatisation to and removal of cyanide have been tested for the treatment of cassava and starch processing wastewaters (Siller and Winter, 1998; Gijzen *et al.* 2000; Annachhatre and Amornkaew 2001). The inhibitory effects of cyanide on the anaerobic process were temporary and reversible. However, at first sight, these practical results seem to contradict the theoretical classification of cyanide as a biocidal inhibitor, i.e. a reactive toxicant whose toxicity is normally irreversible, in the IWA Anaerobic Digestion Model no.1 (ADM1) report (Batstone *et al.*, 2002).

Therefore to clarify this contradiction, the work presented in this paper aimed to study the effect of cyanide, its degradation in anaerobic treatment, extend the ADM1 model with cyanide kinetics and validate the model with experimentation. The experiment uses three upflow anaerobic sludge bed (UASB) lab-scale reactors with sludge adapted to cyanide to test and model the response of the process to different levels of cyanide concentration. For the first time, the different pathways suggested in the literature for cyanide toxicity and inhibition to aceticlastic methanogens and cyanide anaerobic degradation have been formulated in a proposed scenario that is likely to resolve the contradiction between the theoretical classification of the cyanide toxicity and the achieved practical results.

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Materials and methods

Figure 1 shows the updated pathway of ADM1 for the cyanide kinetics. The update to the ADM1 Petersen matrix is listed in Table 1.

Cyanide irreversible toxicity and inhibition

Generally, cyanide is classified as an irreversible enzyme inhibitor and therefore considered toxic (Speece, 1996; Mathews *et al.*, 2000). Applications of anaerobic digestion for the treatment of cassava wastewaters show that, among the anaerobic processes, methanogenesis is the most sensitive to cyanide toxicity (Cuzin and Labat, 1992; Siller and Winter, 1998; Gijzen *et al.*, 2000; Annachhatre and Amornkaew, 2001). In Gijzen *et al.* (2000), sludge activity measurements demonstrated that the effect of CN-inhibition on methanogenic activity was more pronounced for aceticlastic than for hydrogenotrophic methanogens. Therefore modelling the toxicity and inhibition of cyanide will only consider the acetate degraders. Irreversible toxicity of cyanide is applied as a decay factor $I_{dec,Xac,cya}$.

Cyanide inhibition occurs by blocking the active site of enzymes and therefore limits the substrate uptake (Mathews *et al.*, 2000). The inhibition of cyanide is modelled by the inhibition factor I_{cya} to the acetoclastic methanogens.

Reversibility and acclimatisation

Despite the irreversible toxicity of cyanide, a reversible effect to methanogenesis has also been observed in high rate anaerobic reactors after acclimatisation. The reversible effect and acclimatisation can be explained by a population shift from the cyanide sensitive to the cyanide tolerant aceticlastic methanogens. Methanogenic species types and their

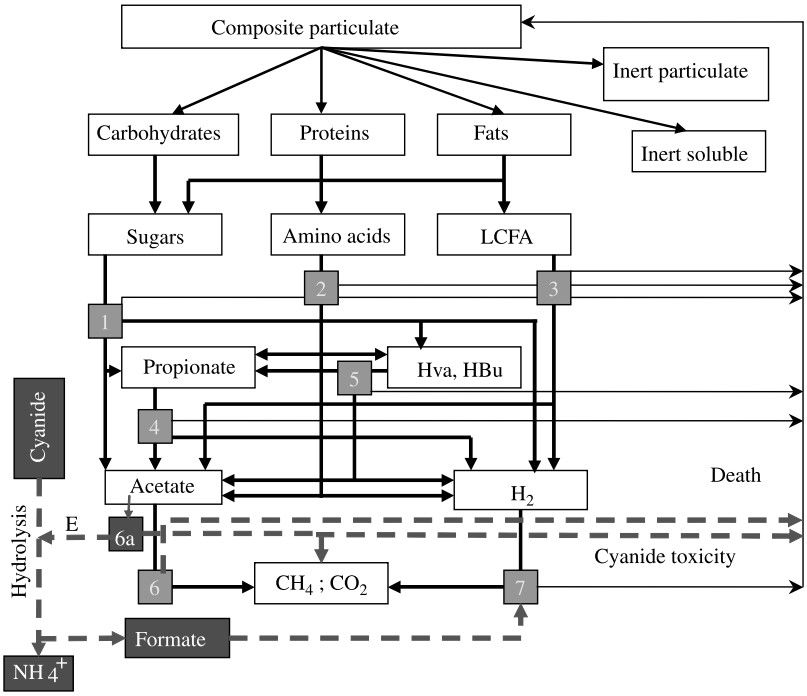

Figure 1 Biochemical processes according IWA ADM1 model (Batstone *et al.*, 2002) extended for cyanide degradation pathway: (6) unadapted aceticlastic methanogenesis; (6a) adapted aceticlastic methanogenesis and cyanide hydrolysis; (7) hydrogenotropic methanogenesis. Dashed lines represents the extension from ADM1

Table 1 ADM1 updated biological reactions matrix

Component i →		7	9	10	11	12a	12b	13	22	22a	23	Rate (ρ_j, kg COD.m^{-3}.d^{-1})
j	Process ↓	S_{ac}	S_{ch4}	S_{IC}	S_{IN}	S_{cya}	S_{fo}	X_c	X_{ac}	$X_{ac,cya}$	X_{h2}	
4a	Hydrolysis of cyanide				1	−1	16					$k_{hyd,cya} \cdot X_{ac,cya} \cdot S_{cya}$
11	Uptake of acetate	−1	$(1 - Y_{ac})$	$-\sum_{i=1-9,11-24} C_i, \nu_{i,11}$	$-(Y_{ac}) \cdot N_{bac}$				Y_{ac}			$k_{m,ac} \cdot \frac{S_{ac}}{K_S + S_{ac}} \cdot X_{ac} \cdot I_4$
11a	Uptake of acetate by cyanide tolerant methanogens	−1	$(1 - Y_{ac,cya})$	$-\sum_{i=1-9,11-24} C_i, \nu_{i,11}$	$-(Y_{ac,cya}) \cdot N_{bac}$					$Y_{ac,cya}$		$k_{m,ac\,cya} \cdot \frac{S_{ac}}{K_S + S_{ac}} \cdot X_{ac,cya} \cdot I_4$
12	Uptake of formate		$(1 - Y_{X_{h2},fo})$	$-\sum_{i=1-9,11-24} C_i, \nu_{i,12}$	$-(Y_{X_{h2},fo}) \cdot N_{bac}$		−1				$Y_{X_{h2},fo}$	$k_{m,fo} \cdot \frac{S_{fo}}{K_{S,fo} + S_{fo}} \cdot X_{h2} \cdot I_3$
18	Decay of X_{ac}							1	−1			$k_{dec,Xac} \cdot X_{ac} \cdot I_{dec,Xac}$
18a	Decay of $X_{ac,cya}$							1		−1		$k_{dec,Xac,cya} \cdot X_{ac,cya} \cdot I_{dec,Xac,cya}$
		Total acetate (kg COD·m^{-3})	Methane gas (kg COD·m^{-3})	Inorganic carbon (k-mole C·m^{-3})	Inorganic nitrogen (k-mole N·m^{-3})	Cyanide concentration (k-mole N·m^{-3})	Formate concentration (kg COD·m^{-3})	Composites (kg COD·m^{-3})	Acetate degraders (kg COD·m^{-3})	Acetate degraders (kg COD·m^{-3})	Hydrogen degraders (kg COD·M^{-3}	Inhibition factors:
												$I_3 = I_{Ph,Xac} \cdot I_{IN,lim} \cdot I_{NH3,Xac}$
												$I_{cya} \frac{K_{I_{cya}}}{K_{I_{cya}} \cdot S_{cya}}$
												$I_4 = I_3 \cdot I_{cya}$
												Decay factors: $I_{dec,Xac} = \frac{S_{cya}}{K_{I_{cya,Xac}}} + 1$
												$I_{dec,Xac,cya} = \frac{S_{cya}}{K_{I_{cya,Xac,cya}}} + 1$

relative population levels in reactor biomass depend on wastewater characteristics as well as on the maintained operational/environmental conditions (Novaes, 1986; Jawed and Tare, 1999). Within the order Methanosarcinales, there are two acetate degrading genera; Methanosaeta (within the order Methanosaetaceae), and Methanosarcina (within the order Methanosarcinaceae). The two genera have different acetate affinity thresholds, and within each genus, member species have different tolerances to temperature, pH, and, importantly in this context, toxic compounds. Therefore, it is possible to have a shift from one species of Methanosaeta to another. Methanosarcina is generally regarded as being more tolerant to toxic compounds than Methanosaeta. However, Zhang *et al.* (2005) found in an UASB treating wastewaters with a high concentration of phenol, that also acts as an irreversible toxicant, that the majority of aceticlastic methanogens were Methanosaeta. This could be explained by their different morphology and granulation properties, which may mean that the different genera are exposed to different toxicant levels. In general, it can be accepted that every species has a different tolerance to toxicants, either because of the resistance of the genus or due to its aggregation and morphology properties.

Enzymatic hydrolysis of cyanide

Fallon *et al.* (1991) observed a strong correlation between microbial activity and cyanide removal in anaerobic digestion and suggested that the removal mechanism depends on microbial activity. They evaluated different anaerobic enrichment cultures in the presence of cyanide. However, it was not possible to find the organism(s) responsible for cyanide metabolism. Cyanide hydrolysis to formate and ammonia is a thermodynamically favourable reaction, $\Delta G^{\circ\prime} = -15.6\,\text{kJ}$. Fallon (1992) reported that the cyanide transformation is analogous to the hydrolysis pathways of aerobes. Hydrolytic reactions are achieved in aerobic systems by enzymes called cyanidases (Raybuck, 1992). Aerobic degradation is carried out by heterotrophs such as *Pseudomonas* that can grow on methanol and acetate as carbon source. Therefore, analogous to aerobic systems, cyanide hydrolysis is modelled as a function of aceticlastic methanogens but is related to the most tolerant group only, $X_{ac,cya}$. The main products from hydrolysis are ammonia and formate: $HCN + 2H_2O \rightarrow HCOOH + NH_3$.

Uptake of cyanide hydrolysis products

Ammonium will be a source of nitrogen for growth. Fallon (1992) suggested that methanogenesis could play a role of removal of the end-products of cyanide hydrolysis so that the cyanide hydrolysis remains thermodynamically favourable. Also, formate degradation is thermodynamically favourable:

$$4HCOOH \rightarrow CH_4 + 3CO_2 + 2H_2O,\ \Delta G^{\circ\prime} = -281\,\text{kJ}$$

All hydrogenotrophic methanogens are capable of utilising formate as substrate for metabolism. Among the acetoclastic methanogens, only *Methanosarcina* is capable of utilising formate for methanogenesis. However, its growth on acetate is inhibited by low concentrations of cyanide (Smith *et al.*, 1985) and, hence, it is not proven that it will become dominant. Therefore, the uptake of formate by hydrogenotrophic methanogens will be used as the main pathway for formate degradation. However, the kinetics used was similar to the ADM1 acetate uptake kinetics but now as function of the hydrogenotrophic biomass since it is a heterotrophic uptake (metabolism of soluble carbon source), i.e. the inhibition terms to formate uptake are considered similar to the acetate uptake.

Table 2 Preliminary parameter estimates of the implemented extension to ADM1

Parameters	Unit	Definition	Estimates
$k_{hyd,cya}$	d^{-1}	Cyanide specific second-order hydrolyses rate	0.6
$k_{m,fo}$	d^{-1}	Monod maximum specific uptake rate of formate	6
$k_{s,fo}$	Kg COD·m^{-3}	Half saturation constant of formate uptake	0.15
$K_{I,cya,Xac}$	kmole·m^{-3}	Inhibition constant to normal acetate degraders decay	3E-005
$K_{I,cya,Xac,cya}$	kmole·m^{-3}	Inhibition constant to tolerant acetate degraders decay	4.7E-004
$K_{I,cya}$	kmole·m^{-3}	Inhibition constant to acetate uptake	1E-004

Experiment and further model updates

Three laboratory-scale UASB reactors (named R1, R2, R3), with 3 L effective volume each, were used to treat cassava starch wastewater with a cyanide concentration of up to 25 mgCN/L. Temperature in the reactors was maintained at 35 °C using a double-jacketed reactor. The organic load per unit of volume of reactors ranged from 3.6 to 11.1 g COD/L.day. All three reactors were seeded with granular sludge from an industrial UASB reactor of a potato chip factory. Sludge is then adapted to the new wastewater in 80–90 days by a stepwise increase in organic load without any addition of external cyanide. By the end of the adaptation period, the three reactors achieved COD removal efficiencies higher than 90%. The cyanide concentration to R3 was increased to 5 mg CN/L on day 99. Then, from day 160, the cyanide load was gradually increased till 14 mg CN/L. R2 received an additional cyanide concentration of 20 mg CN/L starting from day 160. R1 was kept as a control reactor without any addition of external cyanide.

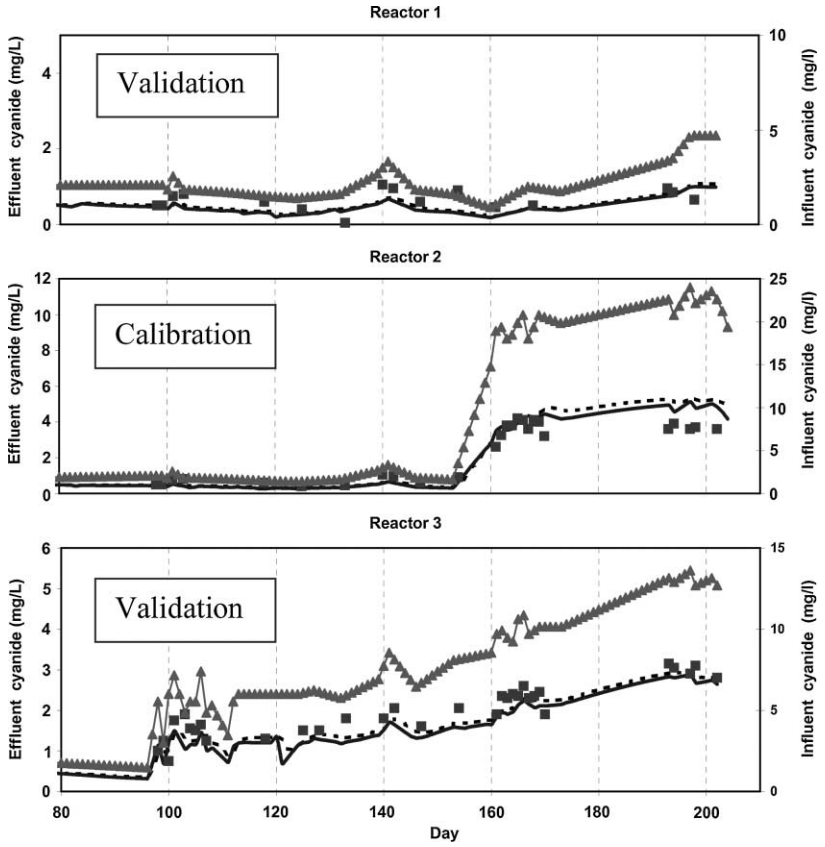

Figure 2 Cyanide simulation results: measurements ■; simulation with WEST implementation —; simulation with AQUASIM implementation; influent cyanide concentration ▲

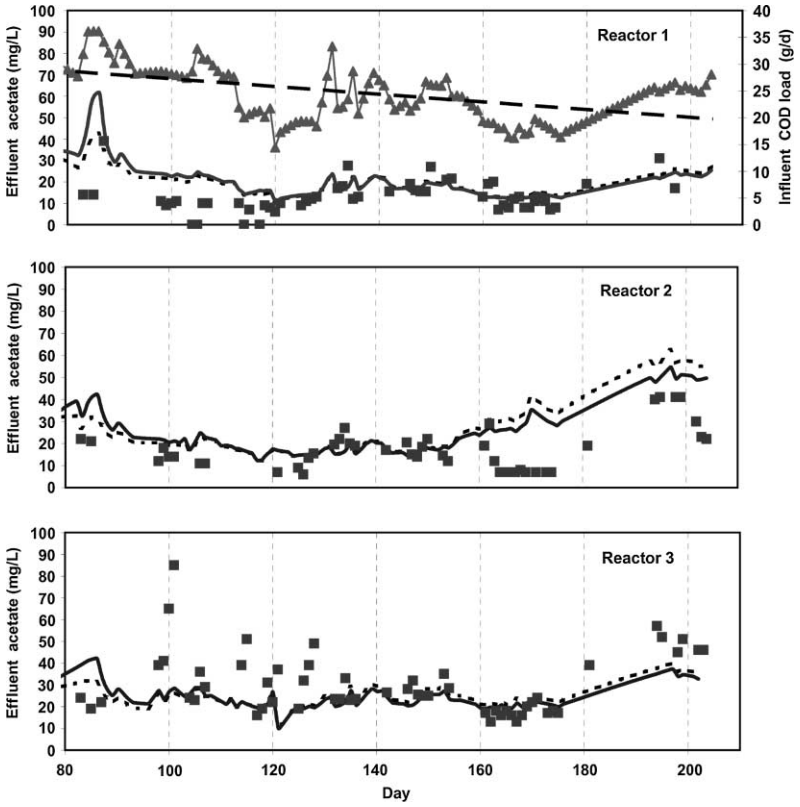

Figure 3 Acetate simulation results: measurements ■; simulation with WEST implementation ; simulation with AQUASIM implementation —; COD load to reactors ▲; trend line of COD load; ——

Carbonate and phosphate buffers and NaOH were added to the wastewater for pH regulation. Therefore, the chemical equilibrium of the ADM1 model had to be extended to consider phosphorus and cyanide buffers. Throughout the experiment, the hydraulic retention time (HRT) in the three reactors was maintained at 12 hours which was enough to maintain a high solids retention time (SRT). The SRT is modelled using a fraction parameter f_{xout} of the particulates leaving the reactor. The parameter f_{xout} has known boundaries between 1 and 0, and is, therefore, better identifiable for ADM1 application to high rate reactors (Zaher *et al.*, 2003).

Implementations and simulation platforms

Two simulation platforms, WEST and AQUASIM, were used for simulation. Two identical implementations of the standard ADM1 on each platform that have previously been validated with dynamic experiments (Zaher *et al.*, 2004) were extended with the above mentioned updates.

Results and discussion

The data set from reactor R2 was used to calibrate the model. Table 2 lists the preliminary parameter estimates of the implemented extension to ADM1.

Data from reactors 1 and 3 were used to validate the model. Although the model was only calibrated on a single step increase of cyanide, experiment R2, the model has been validated on two quite different scenarios. The model is first validated with the normal cyanide level in the influent, experiment R1. The model is then validated further with a

gradual increase in cyanide, experiment R3. Figure 2 shows the cyanide simulation results in the effluent compared to the influent cyanide concentration of the three reactors.

The model could accurately simulate the cyanide concentration in the effluent in response to the low concentration of less than 5 mg CN/l in the feed to R1, the step increase on day 160 from 1.5 to 25 mg CN/l to R2 and the gradual increase from 1.5 to 14 mg CN/l to reactor 3. Both implementations (WEST and AQUASIM) give the same simulation results with slight differences because each platform has different influent interpolation methods.

Figure 3 shows the acetate results in the reactor effluent compared to the COD load to the reactors. The COD load to the three reactors was the same except for a few instances of problems with the feed pumps, mainly on day 86 and on day 120. Recall that the three reactors are fed with the same wastewater, have the same volume and are run to have the same HRT. The main trend of the COD load was gradually decreasing towards the end of the experiment and consequently acetate should have followed the same trend. However, in the three reactors, the acetate concentration was rising because of the introduced cyanide and the model could nicely capture these dynamics. The main kinetics that enabled the model to show these correct responses are the cyanide inhibition terms introduced in the acetate uptake kinetics and the modelled population shift of the acetate degraders.

Still, some differences with the measured data remain. At the end of the experiment, insufficient measurements of acetate in the period 175–195 were available to allow the reliable estimation of the corresponding model parameters. Also, some variations in the acetate measurements for R3 in the period 100–130 could not be captured by the

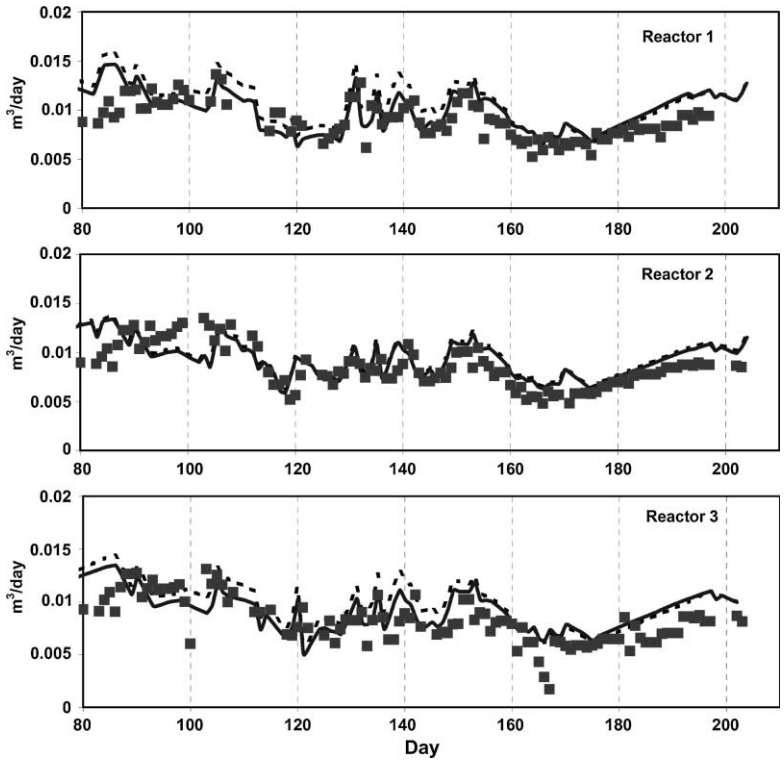

Figure 4 Gas flow simulation results: measurements ■; simulation with WEST implementation —; simulation with AQUASIM implementation .....

model. With further investigation of this period of R3, it was found that the ammonia concentration in the reactor was higher than the normal levels detected throughout the experiment and, therefore, additional ammonia inhibition may have caused acetate accumulation during this period in R3. Also, such effect could be related to some cassava granules that accidentally entered the reactor with the decanted wastewater. Also, in the acetate simulation results there were slight differences between the WEST and AQUASIM.

Figure 4 shows the gas flow results in the three reactors. The gas flow dynamics correspond to the COD load dynamics. The pH in the reactors was kept slightly above 7 and therefore no pH dynamics affect the gas flow. The gas flows from the three reactors are simulated very well by the model except at the points of different inflows due to a fault in the influent pump, e.g. on day 120.

Conclusions

A complete pathway was proposed for the observed anaerobic acclimatisation and degradation of cyanide. Accordingly, kinetics are formulated and extended to the standard IWA ADM1. Anaerobic digestion acclimatisation to cyanide, while being an irreversible toxicant, was explained by modelling a population shift between two acetoclastic methanogens that have a different tolerance to cyanide toxicity. The ADM1 model extended in this work could adequately simulate the process dynamics in three reactors with different cyanide loads. The use of two biomass populations is especially important if selection of a cyanide tolerant biomass is not likely to occur completely.

The modelled hydrolytic pathway for cyanide degradation as function of the proposed cyanide tolerant aceticlastic methanogens concentration helps to accurately simulate the cyanide dynamics in the three reactors, with only one reactor being used for calibration. The introduction of the cyanide inhibition term in the acetate uptake kinetics helped to predict the acetate accumulation during the cyanide overload. After a reasonable acclimatisation period, the biogas in the reactors will follow the influent COD dynamics and can be accurately simulated.

Finally, the ADM1 model with the designed extension could adequately predict the process dynamics in the presence of irreversible toxicity. Hence, the model can be used to study the feasibility of anaerobic treatment of wastewaters contaminated with irreversible toxicants such as cyanide.

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