Comparison Between Acidogenic and Methanogenic Inhibition Caused by Linear Alkylbenzene-Sulfonate (LAS)

J. L. García-Morales*, E. Nebot, L. I. Romero, and D. Sales
Chemical Engineering, Food Technology and Environmental Technology Department, University of Cadiz (Spain).
*Faculty of Marine Sciences (CASEM), Polígono Río San Pedro s/n. 11510, Puerto Real (Cádiz), Spain, e-mail: joseluis.garcia@uca.es

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The anaerobic mixed microbiote may be inhibited through different mechanisms: inhibition by product or substrate, by the physical characteristics of the medium (pH or temperature), and by inhibitory substances, organic or inorganic. Biological activity tests have been the most widely used, of different experimental procedures known, to evaluate the toxicity of various compounds in anaerobic processes. The present study evaluates the inhibitory effect of the anionic surfactant LAS (linear alkylbenzene-sulfonate) on the main anaerobic microbiotes (acidogenic and methanogenic) involved in anaerobic digestion in a reactor treating organic wastewater in the thermophilic range, by means of specific activity tests. Inhibition functions are obtained for inhibition in both microbiotes. The EC_{50} (the concentration that reduces by half the activity of a population) is also calculated, giving EC_{50} (meth)= 6.3 ppm, and EC_{50} (acid)= 18.9 ppm. The results show that the methanogenic microbiote is more sensitive to LAS than the acidogenic.

Key words:
Thermophilic anaerobic digestion, activity determination, LAS

Introduction

Anaerobic purification processes have been shown to be the most suitable treatment for many types of wastewater. These biological processes have been successfully employed, principally, in food industries wastewater with high organic load, in the sludge digestion from urban wastewater treatment plants (WTP), and another industrial wastewater.

Environmental biological processes are subject to multiple fluctuations caused by the variation of parameters such as pH, temperature, organic loading rate (OLR) or concentration of toxic substances or inhibitors. Stability of anaerobic digestion requires the activity of the mixed population of the anaerobic bacteria to be balanced; this stability can be easily disturbed by a variety of factors, causing a rapid increase in the concentration of volatile fatty acids with the concurrent decrease in methane production. When a failure in anaerobic digestion occurs, it is well known that methanogens are the most sensitive members of the anaerobic bacterial consortium.

The anaerobic mixed microbiote may be inhibited through different mechanisms: inhibition by product or substrate, when these compounds are accumulated in the medium, inhibition by the physical characteristic of the medium (pH or temperature), and by inhibitory substances, both organic and inorganic.

The effects on the anaerobic population of many toxic substances have been widely described in the literature. The inhibition by heavy metals[1,2], by cations or anions[3], or by organic substances[4,5] are the most studied. Among organic toxic compounds, the anionic surfactants, such as Linear Alkylbenzene Sulfonate (LAS), have shown inhibitory characteristics in many types of biological treatment process. This compound has shown its inhibitory effect in both aerobic[6] and anaerobic processes[7,8,9].

Anionic surfactants are present nowadays in most detergent formulations and, for this reason, they can be found in a considerable concentration in the urban sewage or in residual waters of some industrial plants. This surfactant can be aerobically biodegraded to higher or lower concentrations depending on the different components in the mixture[10]. In anaerobic conditions, complete biodegradation is not achieved. Several authors think that degradation could occur partially in some of its isomers or homologues[11].

Biological activity tests have been the most widely used, of the various different experimental procedures known, to evaluate the toxicity of various compounds in the anaerobic processes[1,3,12]. The activity of the biomass could be expressed through

two common kinetic parameters: specific substrate consumption ($-q_S$) or specific product generation (q_P)[12], both quantities are easily measured by the test proposed in this work.

The inhibition produced by several agents has been described in the literature by different mathematical functions. These functions relate the observed inhibition to the concentration of the inhibitory agent[13]. Inhibition functions are very useful for the calculation of certain parameters related to the toxicity of the inhibition agents, such as the EC_{50} (the degree of concentration that reduces the activity of a population by half).

The present study evaluates the inhibitory effect of the anionic surfactant LAS on the main anaerobic microbiotes (acidogenic and methanogenic) involved in the anaerobic digestion in a reactor treating organic wastewater in the thermophilic range, through the use of specific activity tests.

Material and methods

Both, acidogenic and methanogenic activity tests were used to show the inhibitory effect of the anionic surfactant LAS (linear alkylbenzene-sulfonate). These tests were used to evaluate the loss of microbiological activity occurring as the concentration of the inhibitor agent (LAS) increases. The procedure consists in incubating an anaerobic biomass sample from the reactor in batch conditions. The rate of activity is determined by the evolution of substrate consumption (glucose or acetate) or product generation (methane). The tests carried out are highly reproducible. An experiment with five identical tests has shown that the standard deviation with respect to the mean maximum activity value is less than 3 %[14].

Equipment and procedures

The anaerobic sludge used was obtained from a laboratory anaerobic Stirred Tank Reactor (STR) treating wine-vinasses in the thermophilic range (55 °C). Capacity of reactor was 5 liters without sludge recycling. The reactor was maintained at the optimum temperature (55 °C) by immersion in a thermostatic bath. The main characteristic of wine-vinasses is its high organic load of about 35 g L^{-1} COD. The feed was supplied to the reactor at one dose per day. The hydraulic retention time (HRT) was maintained at 4 days (dilution rate (D) of 0.25 d^{-1}), this being the optimum HRT obtained in previous studies.

The activity test procedure involves incubating the sludge from a thermophilic lab-scale digester, in sealed anaerobic vials, with a specific substrate according to the microbiological group being tested. For this reason, there is no selection of the microorganism for the activity measurement. Vials of 125 ml with rubber stoppers and crimp seals are used. For specific methanogenic activity tests, the experimental equipment includes a device to measure the generated methane. This device consists of an inverted flask of 250 ml containing an alkaline solution (0.6 mol L^{-1} NaOH or KOH) that allows the selective measurement of methane (see Figure 1).

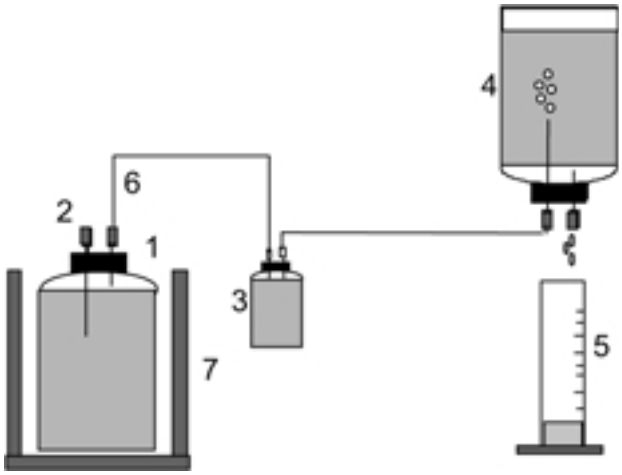

Fig. 1 – *Experimental equipment used to determine methanogenic activity: 1. Assay reactor. 2. Sampling point. 3. Security vessel. 4. Gas-meter. 5. Calibrated cylinder. 6. Biogas bleed-off. 7. Thermostatic bath.*

The anaerobic sludge was obtained by effluent centrifugation at 10,000 g for 15 min. The biomass concentration (X_0) was measured, initially, by analysing the volatile suspended solids concentration (g_{VSS} L^{-1})[15]. A selected medium containing the principal macro and micro-nutrients and the inhibitor agent (LAS), were added to the assay reactor[14].

The centrifuged sludge was added to this solution and then the vial was sealed and flushed for 5 min. with N_2 to obtain anaerobic conditions. In the methanogenic test, the gas-meter was connected at this moment. After this procedure, the vials were immersed in a thermostatic bath (55 °C) for 1 hour, and then the specific substrate – glucose or acetate –, for the acidogenic or methanogenic test, respectively, was added to each assay. Measurements were begun at this moment.

The initial content solution of the linear alkylbencene-sulfonate (LAS) was prepared from a commercial product of the firm Fluka, with a purity of 80–85 % and water mass fraction of less than 5 %.

Experimental plan

The experimental assay conditions: biomass, substrate, and LAS concentration are presented above for each test.

a) For acidogenic activity tests an initial LAS concentration (LAS_0) in the range of 0–50 ppm, biomass concentration (X_0) of 0.18 g_{VSS} L^{-1} and substrate concentration (S_0) of 2.22 ± 0.07 $g_{Glucose}$ L^{-1} (specific substrate for acidogenic microbiote) were added to the reactors.

b) For methanogenic activity test an initial LAS concentration (LAS_0) in the range of 0–10 ppm, biomass concentration (X_0) of 0.51 g_{VSS} L^{-1} and substrate concentration (S_0) of 1500 $mg_{COD(acetate)}$ L^{-1} (specific substrate for methanogenic microbiote) were added to the reactors.

Activity calculation

The activity was defined as the maximum activity attainable. This parametar is the relationship between the maximum rate of consumption of substrate (glucose) or the maximum rate of generation of a product (methane) and the concentration of biomass in g_{VSS} L^{-1}. The units used are g_{Gluc} g_{VSS}^{-1} d^{-1}, for the acidogenic case, and g_{COD} g_{VSS}^{-1} d^{-1} for the methanogenic.

Glucose consumption was assessed from the amount of reducing sugars remaining in each vial[16], this being assessed for the determination of *acidogenic activity.* The evaluation of *methanogenic activity* was determined by measuring the methane produced from acetate used as the methanogenic substrate.

Maximum activity was calculated from experimental results by applying a kinetic model. In this study, the Romero kinetic model[17] was selected. The expression of substrate consumption rate and activity expression for Romero kinetic model are presented in equation 1:

$$(-r_S) = \frac{\mu_{max}}{Y_{X/S}} X_V \frac{(S_t - S_{NB})}{S_{t_0}} = \frac{(r_P)}{Y_{P/S}} \qquad (1)$$

$$A = \frac{(-r_S)}{X_V} = \frac{\mu_{max}}{Y_{X/S}} \frac{(S_t - S_{NB})}{S_{t_0}} \Rightarrow A_{max} = \frac{\mu_{max}}{Y_{X/S}}$$

It could be seen that A_{max} is strongly dependent of μ_{max}.

The Romero kinetic model was used because it is adaptable to a wide range of fermentation processes[10,18] and because, in most cases, it enables some simplifications of the mathematical expression of the model. Another advantage is that all of the experimental data can be used in the calculation. With the Monod model[19], in contrast, only part of experimental data can be used for linear curve fitting[12].

This kinetic model has been widely used to model biological processes[10,18] and to activity calculation[20].

Results and discussion

The experimental protocol was designed to examine the effect of the anionic surfactant (LAS) concentration on the activity of the anaerobic microbiote in the thermophilic range. The Figure 2 shows the development of pH media in the acidogenic activity test versus different LAS concentration, the consumption of glucose in these tests are presented on Figure 3. The methane generation on the methanogenic activity test versus the LAS concentration is shown in Figure 4. The values of LAS concentration (0–10 ppm) used on methanogenic activity test can be found in some industrial and urban residual waters.

The measured values for glucose consumption and methane generation were used to calculate the maximum acidogenic and methanogenic activity values, respectively, by fitting the experimental results to the model by non-linear regression. The results of the calculations are presented in Tables 1and 2.

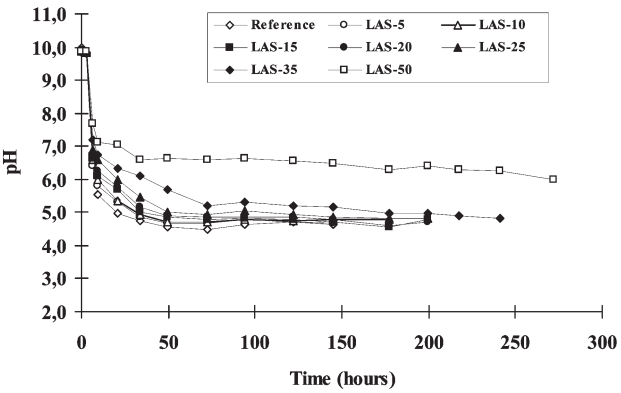

Fig. 2 – *Evolution of media pH in the acidogenic activity tests versus the concentration of inhibitory agent (LAS 0 ppm (reference) to 50 ppm).*

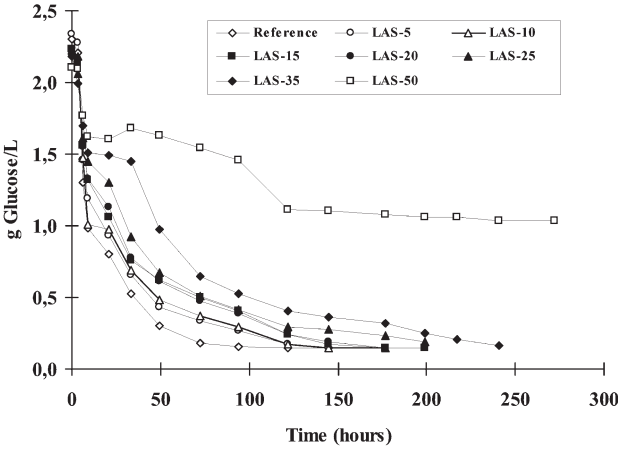

Fig. 3 – *Consumption of glucose in the acidogenic activity test versus the concentration of inhibitory agent (LAS 0 ppm (reference) to 50 ppm).*

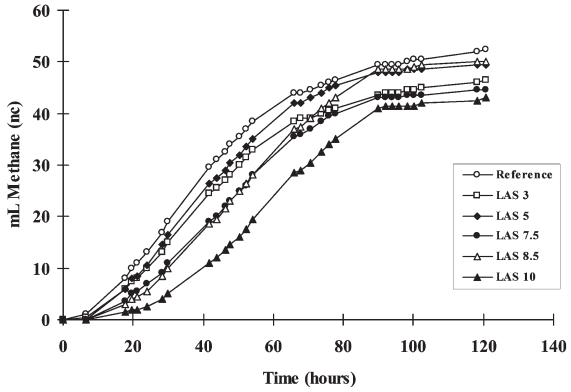

Fig 4. – *Methane generation in the methanogenic activity test versus the concentration of inhibitory agent (LAS 0 ppm (reference) to 10 ppm).*

Table 1 – *Maximum acidogenic activity* A'_{max}. ($g_{Glucose}\ g_{VSS}^{-1} \cdot d^{-1}$)

LAS_0 ppm	A'_{max} $g_{Glucose}\ g_{VSS}^{-1} \cdot d^{-1}$	r^2
0	20.93	0.96
5	17.01	0.96
10	14.98	0.94
15	11.65	0.97
20	10.90	0.97
25	8.78	0.98
35	5.24	0.96
50	2.18	0.86

Table 2 – *Maximum methanogenic activity* A_{max} ($g_{COD\ CH_4}\ g_{VSS}^{-1} \cdot d^{-1}$).

LAS_0 ppm	A_{max} $g_{COD\ CH4}\ g_{VSS}^{-1} \cdot d^{-1}$	r^2
0	0.414	0.99
3	0.292	0.99
5	0.300	0.99
7.5	0.169	0.99
8.5	0.156	0.99
10	0.065	0.99

Analysing the results, it can be observed, that there is, in all of the cases (except in the acidogenic experiment with 50 ppm of LAS), a good fit (r^2) to the experimental data. This model allows all the experimental results to be fitted without previous selection, whereas, the authors who use the Monod kinetic model[19] for activity calculations have to reject those data that do not fit a first- order kinetic approximation of the model [12].

Acidogenic assays

Results for the acidogenic assays show that, when the concentration of the inhibitor (LAS) increases from 0 ppm (reference) to 50 ppm, there is a progressive decrease of the maximum acidogenic activity. In Figure 3 it is observed that when the LAS fraction increases, the incubation period is longer, and a progressive deceleration occurs in the substrate consumption rate in comparison with the reference assay (0 ppm).

The assay evolution in all cases, generally, passes through two phases. The first is a substrate consumption acceleration phase that takes place within the ten first hours. In this phase, the decrease in glucose concentration implies a decrease in pH by volatile fatty acids production. In the second phase, a slower decrease of the substrate consumption rate is registered, until a residual concentration of glucose is reached; this is different for each assay, depending of the initial LAS concentration. The minimum residual value of glucose was measured in the reference assay (0,15 $g_{Glucose}\ L^{-1}$) and maximum value in the LAS-50 (1,46 $g_{Glucose}\ L^{-1}$). In the experiments with different LAS concentration, there is a combined inhibitory effect: the toxic effect generated by the LAS and the inhibitory effect of volatile fatty acid production and the effect of this on the pH of the medium [21].

The form of prevailing inhibition can be deduced from comparison between the different assays and the evolution of the pH of the medium in the reference assay (Figure 2). Thus, in the case of 50 ppm of LAS, for example, it is observed that the inhibition in this assay must be due to the surfactant effect since the pH of the medium (~6.5) is not low enough to be inhibitory. The dependence of the maximum acidogenic activity on the inhibitor concentration can be fitted to the proposed inhibition function presented in equation 2. This function has an exponential form (r^2= 0.99) with the parameters shown in the following equation:

$$A'_{max} = A'_{max_0} \cdot e^{(-\alpha \cdot [LAS])} \qquad (2)$$

– A'_{max_0} = 21.05 ± 0.53 (n = 8, α = 0.05)
– α = 0.037 ± 1.98 · 10^{-3} (n = 8, α = 0.05)

where α shows the acidogenic inhibitory potential per toxic unit (LAS).

This exponential form of the inhibition function is similar to some of those collected by *Bewtra* and *Biswas* (1990) for the inhibition functions of the biological processes in effluents that contain inhibitors or toxic compounds, the fitting results are shown in the Figure 5.

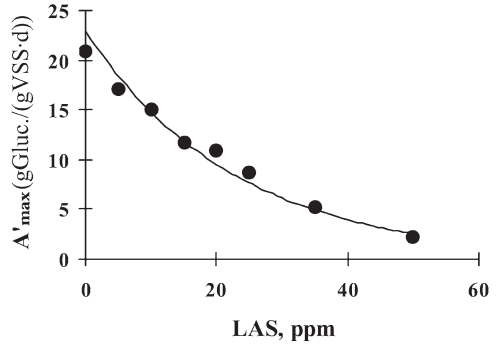

Fig. 5 – *Average value of maximum acidogenic activity, A'_{max_0}, versus LAS concentration, together with the exponential fitting of these variables.*

Methanogenic assays

Results of the methanogenic experiment show that the lowest values of methane production rate were obtained with the greatest concentration of LAS.

In all the assays the existence of an acceleration period of methane production rate (latency phase) is observed ; this is longer in the higher LAS concentration (7.5, 8.5 and 10 ppm). This latency or acclimatization phase (prior to the maximum production rate phase) is indicative of the LAS inhibition effect. The maximum methanogenic activity measurements describe an evolution analogous to the acidogenic case: the activity is less when the LAS fraction in the assay is higher.

The relationship between maximum methanogenic activity and LAS concentration is linear, as shown in the Figure 6.

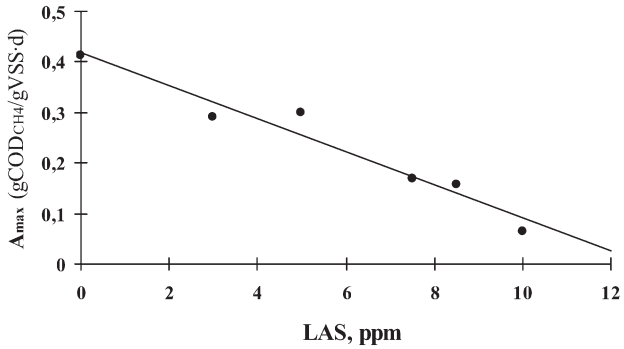

Fig. 6 – *Average value of maximum methanogenic activity, A_{max}, versus LAS concentration, together with the linear fitting of these variables.*

The results of linear fitting of the maximum methanogenic activity and the concentration of the LAS, show a good fit to the experimental results ($r^2 = 0.95$). The linear model is the model that presents a better adjustment. The inhibition function with the fitting quantities are given in the equation below:

$$A_{max} = A_{max_0} + \beta \cdot [\mathrm{LAS}] \qquad (3)$$

– $A_{\max_0} = 0{,}419 \pm 0.026$ ($n = 6$, $\alpha = 0.05$)
– $\beta = 0.033 \pm 3.87 \cdot 10^{-3}$ ($n = 6$, $\alpha = 0.05$)

where β shows the methanogenic inhibitory potential per toxic unit (LAS).

Therefore, the effect of the LAS on the methanogenic population can be considered as a linear inhibition with the inhibitor concentration (LAS). This accords with findings reported in the bibliography[13].

Comparison between the LAS inhibition of acidogenic and methanogenic microbiote

The methanogenic bacteria, in general, are more sensitive to environmental changes and to the presence of toxic compounds or inhibitors than the acidogenic bacteria (Figure 7). The acidogenic population, at the same time, can suffer inhibition by the accumulation of the final product like volatile fatty acids or H_2. These products are potential substrates for the methanogenic population. For this reason, an inhibition of the methanogenic population produces in a short period of time an inhibition of the acidogenic population and the imbalance of the process.

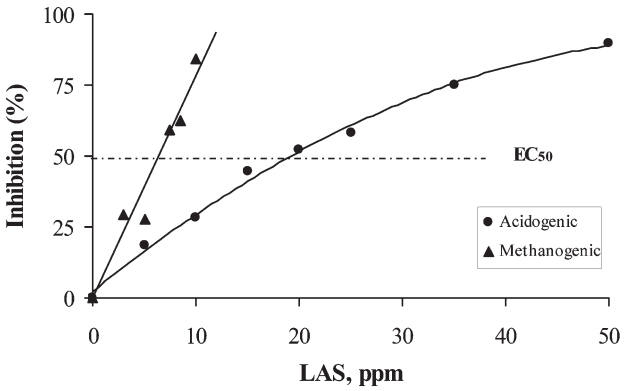

Fig. 7. – *Evolution of inhibition percentage (%) versus LAS concentration. Values of EC_{50} for each microbiote.*

The obtained results indicate a decrease of almost 85 % in the maximum methanogenic activity, comparing the reference with the assay with the highest fraction of the inhibitor (10 ppm). This surfactant concentration only produces a decrease of 29 % in the maximum acidogenic activity. Expressing these results using a parametar more usual in inhibition studies, EC_{50}, (the concentration that reduces the maximum activity of the population by half), it is found that EC_{50} (methanogenic) = 6.3 ppm and EC_{50} (acidogenic) = 18.9 ppm. These values of LAS concentration can be found in some industrial and urban residual waters.

In the acidogenic case, the decrease of the maximum acidogenic activity between the reference and the highest LAS concentration tested, 50 ppm, is more than 89 %.

Conclusions

The main conclusions of the study are the quantification of the inhibitory character of LAS on the different microbiotes involved in anaerobic thermophilic digestion, and the validity of the proposed tests for the determination of this inhibition.

The LAS inhibition in anaerobic systems can be associated with two closelyinterrelated causes. The first is the direct inhibition by the surfactant, which is shown in the methanogenic as well as in the acidogenic cases. The second cause is the consequence of the imbalance produced in the syntrophic relationship existing between the different anaerobic populations.

The forms of the inhibition functions, that describe the relationship between the maximum activity and the concentration of the toxic substance, are similar to those described in the bibliography by different authors [13].

The thermophilic anaerobic systems studied, from laboratory reactors treating a wine distillery wastewater, were not adapted to this toxic substance. It may be supposed that after an period of adaptation to the toxic substance, there could be a decrease of the inhibitory effect of the LAS on this population.

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Nomenclature

A – activity

A_{max} – maximum methanogenic activity, $g_{COD\ CH4}\ g_{VSS}^{-1} \cdot d^{-1}$

A_{max_0} – maximum methanogenic activity without inhibitor agent, $g_{COD\ CH4}\ g_{VSS}^{-1} \cdot d^{-1}$

A'_{max} – maximum acidogenic activity, $g_{Gluc.}\ g_{VSS} \cdot d^{-1}$

$A'_{max\,0}$ – maximum acidogenic activity without inhibitor agent, $g_{Gluc.}\ g_{VSS} \cdot d^{-1}$

COD – chemical oxygen demand

LAS_0 – linear alquilbenzene sulphonate initial concentration, LAS (ppm, mg L^{-1} LAS)

$(-r_s)$ – Net rate of substrate consumption – Romero Kinetic model, g L^{-1} d^{-1}

(r_P) – Net rate of product generation – Romero Kinetic model, g L^{-1} d^{-1}

S – substrate, g L^{-1}

S_{NB} – non-biodegradable substrate concentration – Romero Kinetic model – g L^{-1}

S_0 – initial concentration of substrate, g L^{-1}

VSS – volatile suspended solids, g L^{-1}

X_V – Active micro-organisms concentration – Romero Kinetic model – g L^{-1}

X_0 – biomass initial concentration, g_{VSS} L^{-1}

$Y_{P/S}$ – yield coefficient product/substrate – Romero Kinetic model

$Y_{X/S}$ – yield coefficient microorganism / substrate – Romero Kinetic model –

α – fitting parameter of A'_{max} to the exponential function, L mg^{-1} LAS

β – fitting parameter of A_{max} to the linear function, $(g_{COD\ CH4}) \cdot L^{-1} / (g_{VSS} \cdot d^{-1} \cdot mg^{-1}$ LAS)

μ_{max} – maximum specific grown rate of the microorganism – Romero Kinetic model, d^{-1}

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