Microbial colonization of anaerobic biofilms: a mathematical model

B. D'Acunto[1], G. Esposito[2], L. Frunzo[1], V. Luongo[1,3,*], M.R. Mattei[1], F. Pirozzi[3]

[1] Department of Mathematics and Applications Renato Caccioppoli,
University of Naples *Federico II*, via Cintia, Monte S. Angelo, I-80126 Naples, Italy.
[2] Department of Civil and Mechanical Engineering, University of Cassino and Southern Lazio,
via Di Biasio 43, 03043 Cassino (FR), Italy.
[3] Department of Civil, Architectural and Environmental Engineering,
University of Naples *Federico II*, via Claudio 21, 80125 Naples, Italy.

* Corresponding author: E-mail: vincenzo.luongo@unina.it,
Tel +39 081 7683436, Fax: +39 081 5932344

Abstract

A 1-D mathematical model for analysis and prediction of microbial colonization of anaerobic multispecies biofilms for methane production is presented. The model combines the related processes of hydrolysis, acidogenesis, acetogenesis, methanogenesis and takes into account phenomena of substrate reaction and diffusion, biomass growth, detachment and, in particular, the colonization of new species from bulk liquid to biofilm. The colonization phenomenon is initiated by planktonic cells, present in the bulk liquid but not initially in the biofilm, which thanks to the characteristic porous structure of biofilm matrix, may enter the channels and establish where they find favorable growth conditions. The model consists of a free boundary value problem where the biofilm growth process is governed by nonlinear hyperbolic PDEs and substrate dynamics are dominated by semilinear parabolic PDEs. The transport of colonizing bacteria from the bulk liquid to the biofilm is modelled by using a diffusion-reaction equation, where the reaction term represents the loss of planktonic bacteria due to their establishment within the biofilm. The method of characteristics is used for numerical purposes. The model is based on the biological framework of ADM1 and has been applied to simulate microbial competition and evaluate the influence of substrate diffusion on microbial stratification. Specific scenarios have been simulated describing the effect of colonization of motile bacteria into an established anaerobic biofilm.

Keywords: anaerobic digestion; biofilms; invasion model; nonlinear PDEs; method of characteristics

1. INTRODUCTION

Anaerobic digestion (AD) process has been used for over a century for the effective treatment of organic wastes and it is currently recognized as one of the major treatment technologies for solid wastes and wastewaters. The interest in anaerobic treatment is increasing over years as it presents some significant advantages, such as the biological

production of a methane rich gas flow, when compared to the alternative aerobic treatments. Indeed, AD has been effectively recognized as an eco-friendly, cost-efficient and low-energy required technology [1-2].

The AD of complex organic substrates is generally achieved through the sequential and coordinated activity of various microbial groups, which catalyze three main reactions: hydrolysis, acid fermentation and methanogenesis [3]. These microbial groups establish syntrophic relationships revealing in many cases in metabolic cooperation, with one species utilizing the product of a coexistent species [4]. One of the main limitations of the conventional AD reactors relies on the different growth rates of such various microbial groups, that influence each other with producing specific compounds that can be utilized by other species or can be inhibitory for the process. Methanogenesis, for example, involves slow-growing micro-organisms and it is generally considered as the limiting reaction in the anaerobic digestion process. The failure of full-scale anaerobic digesters is often linked to low activity of methanogenic archaea [5]. Moreover, this microbial group is extremely sensitive to any disturbance in anaerobic digesters, such as organic overload, leading to inhibitory working pH and accumulation of organic acids and H_2 [6].

As widely demonstrated by experimental evidence, the microbial community interactions are strongly affected by various operating and designing conditions, such as the pH value, the operating temperature, the composition of the feedstock, the organic loading rate and the hydraulic retention time. For instance, different microbial communities develop in digesters operating on different retention times as the choice of a shorter value may even lead to the washout of the slow growing methanogens, which on their count require more time to ensure complete degradation of organic matter in conventional anaerobic digesters [7]. This drawback is usually overcome with increasing the reactor volume and the designed hydraulic retention time.

Considerable effort has been recently devoted to the development of high rate reactors with decreasing reactor volume or retention time to maximize community functions and the related methane production [8]. Even though the intensified practice of AD has led to many different modifications to the conventional reactor configurations, anaerobic biofilm reactors represent one of the most promising technologies in the field of high rate digesters [9]. In these systems, microorganisms grow attached to an inert solid surface and/or each other forming micro colonies or biofilms. The adhesion of microorganisms over solid carriers with large specific surface areas, leads to high biomass concentration and high reaction rates, thus reducing the reactor volume needed [10]. Moreover, the grow of bacteria in the sessile state makes possible decoupling the hydraulic retention time from the residence time of the biomass. On the other hand, these systems are characterized by long start-up periods mainly related to the slow spontaneous development and maturation of the biofilm [11-12]: these might take several months to obtain an active and stable biofilm [13].

According to [14], the development of an anaerobic multispecies biofilm can be divided into three stages: an initial attachment phase characterized by random adhesion of the cells to the inert surface; a consolidation phase defined by the appearance of microcolonies also defined as irreversible attachment and characterized by producing extracellular polymeric substances (EPS); and a maturation phase [13-15]. The complexity of the microbial ecosystem has been found to increase over time due to different abiotic and biotic conditions: hydrodynamic conditions [16], types and nature

of carrier material [5], substrate composition [17], source of inoculum [5] and availability of trace metals [18-19]. Indeed, the appearance of new community members in the structure of the multispecies biofilm is probably due to the accumulation of metabolic waste products, such as the acetate for acetogenic bacteria, that can be used as growth substrates by the new colonizer microorganisms, i.e. methanogenic bacteria. The latter show a reduced capability of colonizing the surface but their establishment within the biofilm is strongly affected by the formation of favorable environmental conditions for their growth. The presence of relatively large channels and pores within the matrix structure might allow the entry of these colonizing cells which may abandon the planktonic state and start to grow as biofilm.

In parallel to experimental investigations, complex mathematical models and numerical simulations have been proposed to investigate development, structures, and ecological interactions of anaerobic biofilms. However, little attention has been directed towards successional invasion in anaerobic biofilms. Here a mathematical model for anaerobic multispecies biofilm formation and development based on the biological framework of ADM1 is presented.

Numerical simulations demonstrate the capability of the model to predict biomass distribution, substrate concentration profiles within the biofilm, and the invasion of new bacterial species. In particular, simulation results illustrate the dynamics and evolution of archaea colonization of an anaerobic biofilm constituted initially by fermentative bacteria.

2. MATHEMATICAL MODEL

The mathematical model has been formulated as a hyperbolic free boundary value problem in the framework of continuum modelling of biofilm growth. It takes into account the dynamics of a multispecies biofilm, constituted by *n* microbial species assumed to cover homogeneously the support surfaces of a biofilm reactor. Some species are defined as *resident* and are supposed to initially inhabit the biofilm. Conversely, the *invading species* are not present initially in the biofilm but their growth relies on the presence of planktonic cells, which are able to diffuse from the bulk liquid to the biofilm and switch their mode of growth from suspended to sessile when appropriate environmental conditions are found. The biofilm expansion is regulated by some growth limiting nutrients which are dissolved in the bulk liquid, whose dynamics have been considered as well. The mathematical problem has been derived by coupling the mass balance equations for substrates within the bulk liquid with a full one-dimensional invasion biofilm model as presented in [17] and [20]. The model is formulated for the variables: concentration of microbial species in sessile form, X_i, concentration of planktonic colonizing cells, Ψ_i, concentration of dissolved substrates within the biofilm S_j, all expressed as functions of time t and z, which denotes the spatial coordinate assumed perpendicular to the substratum. The variable S^*_j denotes the concentration of substrate *j* within the bulk liquid. The model equations take the following form:

$$\frac{\partial X_i}{\partial t} + \frac{\partial}{\partial z}(uX_i) = \rho_i r_{Mi}(z,t,\mathbf{X},\mathbf{S}) + \rho_i r_i(z,t,\mathbf{\Psi},\mathbf{S}), \ 0 \leq z \leq L(t), \ t > 0, \ i = 1,...,n, \quad (1)$$

244

Proceedings
of the Sixth International Conference
on Environmental Management, Engineering, Planning & Economics
Thessaloniki, Greece, June 25-30, 2017
ISBN: 978-618-5271-15-2

$$\frac{\partial u}{\partial z} = \sum_{i=1}^{n} (r_{Mi} + r_i), \ 0 < z \le L(t), \ t \ge 0, \tag{2}$$

$$\frac{\partial \Psi_i}{\partial t} - D_{Mi} \frac{\partial^2 \Psi_i}{\partial z^2} = r_{\Psi,i}(z,t,\mathbf{\Psi},\mathbf{S}), \ 0 < z < L(t), \ t > 0, \ i = 1,...,n, \tag{3}$$

$$\frac{\partial S_j}{\partial t} - D_j \frac{\partial^2 S_j}{\partial z^2} = r_{S,j}(z,t,\mathbf{X},\mathbf{S}), \ 0 < z < L(t), \ t > 0, \ j = 1,...,m, \tag{4}$$

$$V\dot{S}_j^* = -AD_j \frac{\partial S_j}{\partial z}(L(t),t) + Q(S_j^{in} - S_j^*(t)), \ t > 0, \ j = 1,...,m, \tag{5}$$

$$\dot{L}(t) = u(L(t),t) + \sigma(t), \qquad t > 0, \tag{6}$$

where:

$X_i(z,t) = \rho_i f_i$ denotes the concentration of the microbial species i $\mathbf{X} = (X_1,...,X_n)$; ρ_i is the constant biofilm density; f_i denotes the volume fraction of microbial species; $S_j(z,t)$ is the concentration of substrate j; $\psi_i(z,t)$ represents the concentration of planktonic cells i diffusing from bulk liquid to biofilm, $\psi = (\psi_i,...,\psi_n)$; $u(z,t)$ is the velocity of the microbial mass displacement with respect to the biofilm support interface; D_j denotes the diffusivity coefficient of substrate j; D_{Mi} denotes the diffusivity coefficient of planktonic species i; $r_{Mi}(z,t,X,S)$ is the specific growth rate of the sessile species, $r_i(z,t,\psi,S)$ is the specific growth rate due to the colonization process; $r_{\psi i}(z,t,\psi,S)$ is the loss term of planktonic cells due to the colonization phenomenon; $r_{S,j}(z,t,X,S)$ is the conversion rate of substrate j; $\sigma(t)$ is the exchange flux between biofilm and bulk liquid. In addition, V, Q and A denote the volume, inlet and outlet flow rate and surface area of the biofilm reaction, assumed to be fed with a constant inlet substrate concentration S_j^{in}. The following initial-boundary conditions are considered for Eqs (1)-(6):

$$X_i(z,0) = \begin{cases} \varphi_i(z), i = 1,..,n_1 \\ \varphi_i(z) = 0, i = n_1 + 1,..,n \end{cases}; \quad u(0,t) = 0, \quad 0 \le z \le L_0, \ t \ge 0, \ i = 1,...,n, \tag{7}$$

$$\frac{\partial \Psi_i}{\partial z}(0,t) = 0, \ \Psi_i(z,0) = \Psi_{0i}(z) = 0, \ \Psi_i(L(t),t) = \Psi_{iL}(t), \quad 0 \le z \le L_0, \ t > 0, \ i = 1,...,n,$$

$$S_j(z,0) = S_{0j}(z), \ S_j^*(0) = S_j^{in}, \quad 0 \le z \le L_0, \ j = 1,...,m,$$

$$\frac{\partial S_j}{\partial z}(0,t) = 0, \ S_j(L(t),t) = S_j^*(t), \ t > 0, \ j = 1,...,m, \tag{8}$$

$$L(0) = L_0.$$

The functions $\phi_i(z)$ represent the initial concentrations of biomass species i, the functions $S_{0i}(z)$ represent the initial substrate concentrations within the biofilm, the functions $\psi_{iL}(t)$ represent the concentration of the planktonic cells in the bulk liquid. Note that n_1 denotes the number of resident species. Moreover, the number of planktonic species is assumed equal to the number of sessile species n to make the model general.

Proceedings

245

of the Sixth International Conference
on Environmental Management, Engineering, Planning & Economics
Thessaloniki, Greece, June 25-30, 2017
ISBN: 978-618-5271-15-2

The rate terms $r_{Mi}(z,t,X,S)$ describing the growth of sessile cells, are controlled by the local availability of nutrients and usually described as standard Monod kinetics. They also account for the natural cell death. The terms $r_i(z,t,\psi,S)$, represent the growth rates of the microbial species X_i due to the colonization process and are assumed as a function of the planktonic cells and substrate concentrations. They both need to be defined based on the biological case to be modelled.

3. NUMERICAL APPLICATION

The model has been applied to simulate the invasion of methanogenic archaea in an anaerobic biofilm operating in a continuous reactor. The analysed scenario describes the dynamics of two microbial species, the fermentative bacteria X_1 and the methanogenic archaea X_2, whose initial concentration within the biofilm has been set to zero.

Table 1. Kinetic-stoichiometric parameters and initial-boundary conditions.

Symbol	Definition	Value	Unit
Y_1	Yield on X_1	0.1	-
Y_2	Yield on X_2	0.05	-
$\mu_{max,1}$	Maximum growth rate of X_1	30	d^{-1}
$\mu_{max,2}$	Maximum growth rate of X_2	5	d^{-1}
Ks_1	S_1 affinity constant for X_1	0.5	$gCOD\ L^{-1}$
Ks_2	S_2 affinity constant for X_2	0.15	$gCOD\ L^{-1}$
kd_1	Decay rate of X_1	0.005	d^{-1}
kd_2	Decay rate of X_1	0.005	d^{-1}
Y_ψ	Yield of X_2 on Ψ_2	0.01	-
k_{col}	Maximum colonization rate of Ψ_2	0.001	d^{-1}
k_Ψ	Kinetic constant for Ψ_2	0.01	$mgCOD\ L^{-1}$
φ_1	Initial volume fraction of X_1	1	-
φ_2	Initial volume fraction of X_2	0	-
S_1^{in}	Inlet concentration of S_1	6	$gCOD\ L^{-1}$
S_2^{in}	Inlet concentration of S_2	0	$gCOD\ L^{-1}$
L_0	Initial biofilm thickness	0.01	mm

The model takes into consideration three reactive components, dissolved organic matter S_1, acetate S_2 produced by X_1 and consumed by X_2 and methane S_3, which represents the final product of the whole metabolic pathway. According to experimental evidence, the establishment and proliferation of X_2 in sessile form depends on the formation of a favourable environmental niche, which corresponds to the accumulation of acetate. The corresponding substrate concentrations within the bulk liquid $S_j*(t)$ have been taken into account as well. Planktonic cells have been considered for X_2. The

246

Proceedings
of the Sixth International Conference
on Environmental Management, Engineering, Planning & Economics
Thessaloniki, Greece, June 25-30, 2017
*ISBN: 978-618-5271-15-2*

reaction rates in Equations (1), (3) and (4) have been defined according to ADM1. The initial biofilm thickness of 0.01 mm has been assumed and the concentration of colonizing archaea in the bulk liquid has been set to $\psi_2(L(t),t)=0.1$ mg COD L^{-1}. Table 1 resumes all the kinetic and stoichiometric parameters and the initial and boundary conditions adopted, such as the concentrations of soluble substrates in the inlet flow rate and the initial biofilm composition used for the specific simulation. Numeric integration of the system (1)-(6) has been performed using the software tool MATLAB$^{®}$. The numerical method has been based on the method of characteristics.

4. RESULTS AND DISCUSSION

The simulation reproduces the archaea colonization phenomenon and tracks the dynamics of the bacterial species and the evolution of substrate profiles within the biofilm. The simulation is reported in Figure 1 to Figure 3. In particular, the results are expressed in terms of bacterial volume fractions (A), substrate concentration profiles (B) and methane production (C) at different simulation time (note that biofilm is growing from left to right in all the Figures). The methane production is expressed as the percentage of the maximum theoretical production.
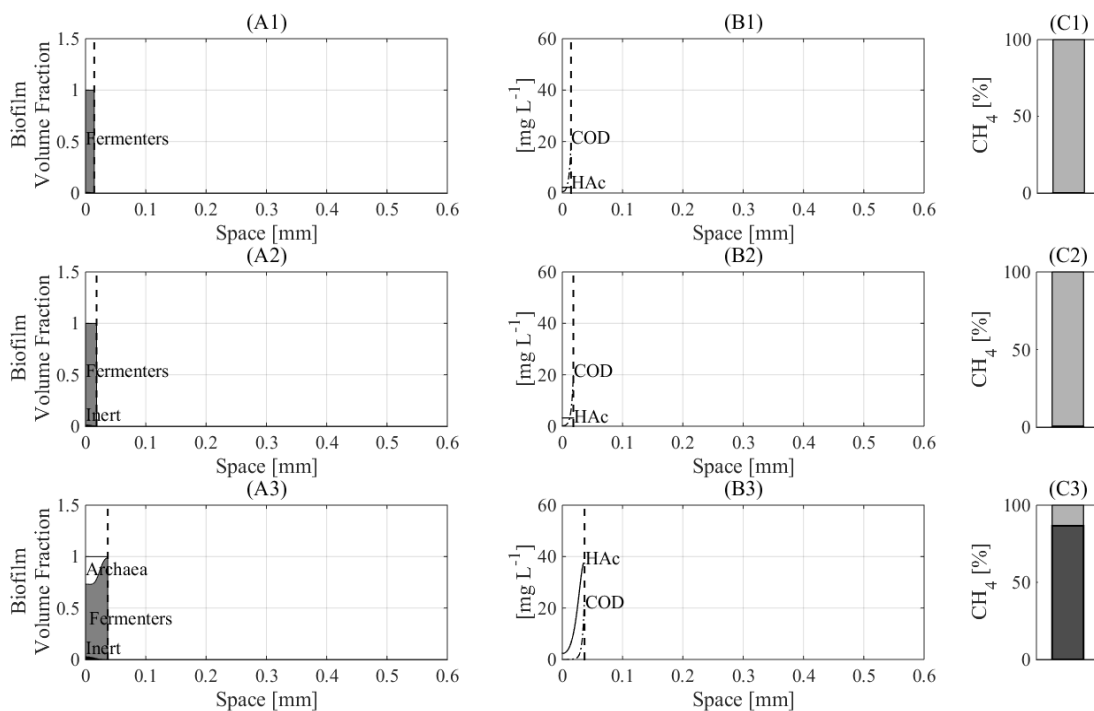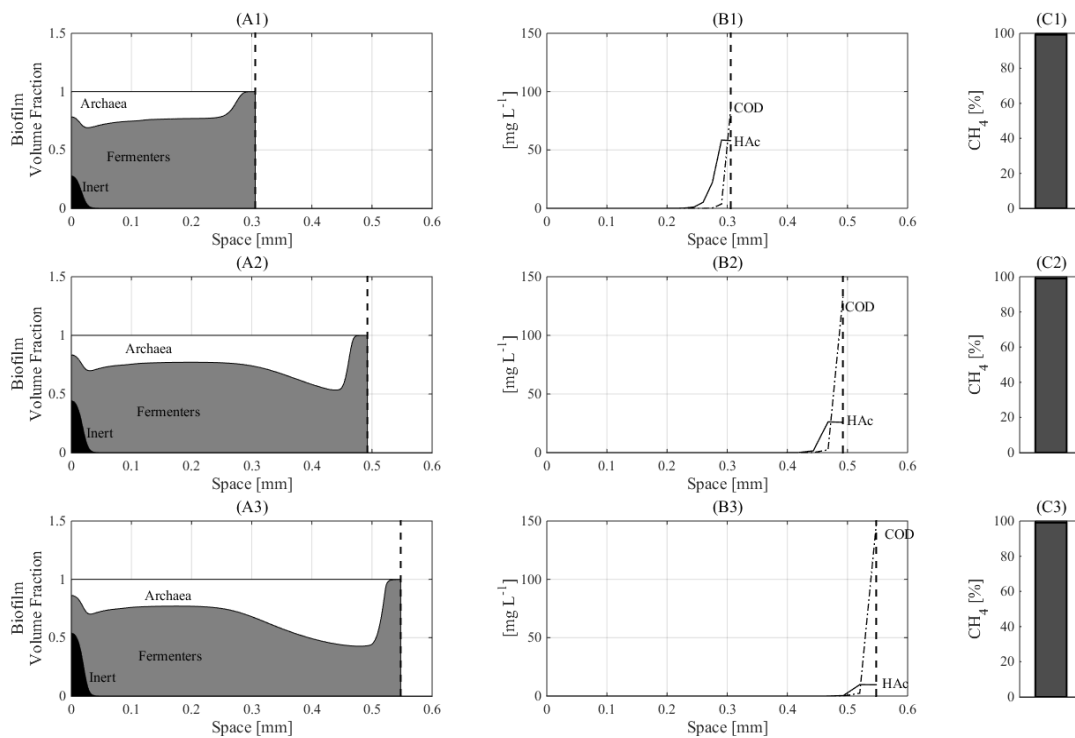

Figure 4 Bacteria volume fractions, substrate concentration trends and substrate to methane conversion efficiency, after 1 (A1, B1, C1), 2 (A2, B2, C2) and 5 (A3, B3, C3) days.
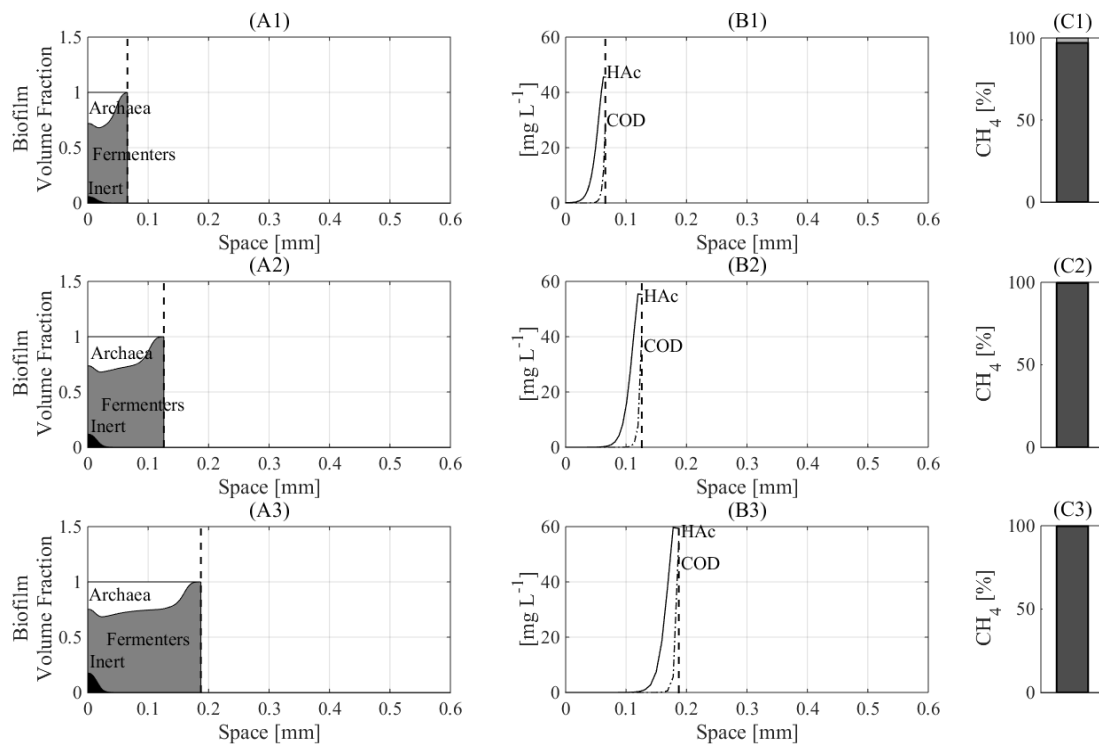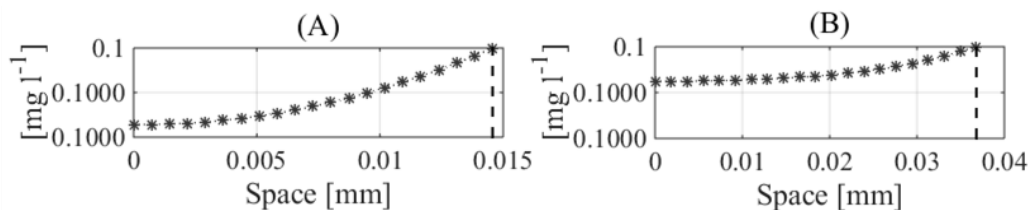
Figure 5 Bacteria volume fractions, substrate concentration trends and substrate to methane conversion efficiency, after 10 (A1, B1, C1), 20 (A2, B2, C2) and 30 (A3, B3, C3) days.

The numerical results show that colonizing bacteria diffuse into the biofilm and grow only where there are favorable environmental conditions for their development (Figure 2 (A1, A2, A3)), as determined by substrates trends (Figure 1 (B3) and Figure 2 (B1, B2, B3)). According to experimental evidence, the archaea cells colonize the inner part of the biofilm, where they found favourable environmental conditions, that is high acetate and low dissolved organic matter concentrations. Under these conditions, archaea can effectually prevail on the fermentative bacteria. During the first days of simulation time, the production of methane is equal to zero due to the absence of methanogenic biomass within the biofilm. As expected, acetic acid shows a constant profile when archaea are not present within the biofilm (Figure 1 (B1, B2)). As soon as archaea start to grow (Figure 1 (A3)), the acetate concentration sharply decreases, (Figure 1 (B3)). The colonization phenomenon completely evolves in Figures 2 and 3. Specifically, the acetate concentration reduces significantly all over the biofilm and a residual dissolved organic matter is found in the bulk liquid (Figure 3 (B1, B2, B3)). Contextually, the maximum methane yield is definitely achieved (Figure 3 (C1, C2, C3)).

248

Proceedings
of the Sixth International Conference
on Environmental Management, Engineering, Planning & Economics
Thessaloniki, Greece, June 25-30, 2017
ISBN: 978-618-5271-15-2

Figure 6 Bacteria volume fractions (A), substrate concentration trends (B) and substrate to methane conversion efficiency, after 50 (A1, B1, C1), 90 (A2, B2, C2) and 120 (A3, B3, C3) days.

Finally, it is important to notice that the diffusion of mobile colonizing archaea into the biofilm allows the colonization of a new species as determined by substrate (i.e. acetic acid) profiles. More precisely, as shown in Figure 4, the biofilm results fully penetrated by ψ_1, which never reaches zero, indicating that merely the contemporary presence of substrates and colonizing motile species can lead to the growth of sessile bacteria. Despite, the concentration of the invading species is higher in the external part of the biofilm, the archaea growth occurs in the inner part of the biofilm where the environmental conditions are more favorable. This fact is consistent with observations that substrate concentrations have a regulatory effect on the dynamics of biofilm structure since the colony size can be directly correlated with the substrate concentration profiles into the biofilm [17].

Figure 4 ψ profile within biofilm after 1 (A), and 120 (B) days.

Proceedings
of the Sixth International Conference
on Environmental Management, Engineering, Planning & Economics
Thessaloniki, Greece, June 25-30, 2017
ISBN: 978-618-5271-15-2

249

5. CONCLUSIONS

A mathematical model describing the invasion of methanogenic archaea into an already constituted anaerobic biofilm has been presented. The mathematical modeling of the invasion phenomenon applied to anaerobic digestion systems results extremely important to clarify the community functions of these complex systems and the mechanisms regulating the methane production in anaerobic biofilm reactors. In the numerical simulation, a well-known example has been developed. The results show that the model is able to predict the colonizing process in a reasonable way. The model predictions will help engineers or operators to have a better insight into biofilm dynamics allowing the optimization of process designing or practical operation.

REFERENCES

[1] Aydin, S., Ince, B., Ince, O., 2015. Application of real-time PCR to determination of combined effect of antibiotics on bacteria, methanogenic archaea, archaea in anaerobic sequencing batch reactors. *Water Research*, **76**, 88-98.

[2] Minale, M., Worku, T., 2014. Anaerobic co-digestion of sanitary wastewater and kitchen solid waste for biogas and fertilizer production under ambient temperature: waste generated from condominium house. *International Journal of Environmental Science and Technology*, **11**, 509-516

[3] McKeown, R.M., Hughes, D., Collins, G., Mahony, T., O'Flaherty, V., 2012 Low-temperature anaerobic digestion for wastewater treatment. *Current opinion in Biotechnology*, **23**, 441-451.

[4] Garcia, J.-L., Patel, B.K.C., Ollivier, B., 2000. Taxonomic, Phylogenetic, and Ecological Diversity of Methanogenic Archaea. *Anaerobe*, **6**, 205-226.

[5] Habouzit, F., Hamelin, J., Santa-Catalina, G., Steyer, J.P., Bernet, N., 2014. Biofilm development during the start-up period of anaerobic biofilm reactors: the biofilm archaea community is highly dependent on the support material. *Microbial Biotechnology*, **7**, 257-264.

[6] Leitão, R.C., Van Haandel, A.C., Zeeman, G. and Lettinga, G., 2006. The effects of operational and environmental variations on anaerobic wastewater treatment systems: a review. *Bioresource Technology*, **97** (9), 1105-1118.

[7] Poh, P.E., Chong, M. F., 2009. Development of anaerobic digestion methods for palm oil mill effluent (POME) treatment- A Review. *Bioresource Technology*, **100**,1–9.

[8] Rajeshwari K. V., Balakrishnan, M., Kansal, A., Kusum, L., Kishore, V.V.V., 2000. State-of-the-art of anaerobic digestion technology for industrial wastewater treatment. *Renewable and Sustainable Energy Reviews*, **4**,135-156.

[9] Yeshanew, M.M, Frunzo, L, Luongo, V., Pirozzi, F., Lens, P.N.L., Esposito, G., 2016. Start-up of an anaerobic fluidized bed reactor treating synthetic carbohydrate rich wastewater. *Journal of Environmental Management*, **184**, 456-464.

[10] Wang, W., Hou, H., 2010. Performance and stability improvements in anaerobic digestion of thermally hydrolyzed municipal biowaste by a biofilm system. *Bioresource Technology*, **101** (6), 1715-1721.

[11] Cresson, R., Escudie, R., Steyer, J.P., Delgenes, J.P., Bernet, N., 2008. Competition between planktonic and fixed microorganisms during the start-up of methanogenic biofilm reactors. *Water Research*, **42** (3), 792-800.

[12] Encina, P.A.G., Hidalgo, M.D., 2005. Influence of substrate feed patterns on biofilm development in anaerobic fluidized bed reactors (AFBR). *Process Biochemistry*, **40** (7), 2509-2516.

[13] Escudie, R., Cresson, R., Delgenes, J.P., Bernet, N., 2011. Control of start-up and operation of anaerobic biofilm reactors: an overview of 15 years of research. *Water Resources*, **45** (1), 1-10.

[14] Fernández, N., Díaz, E.E., Ricardo Amils, R., Sanz, J.L., 2008. Analysis of Microbial Community during Biofilm Development in an Anaerobic Wastewater Treatment Reactor. *Microbial Ecology*, **56**, 121-132.

[15] Bialek, K., Kumar, A., Mahony, T., Lens, P.N.L., O'Flaherty, V., 2012. Microbial community structure and dynamics in anaerobic fluidized-bed and granular sludge bed reactors: influence of operational temperature and reactor configuration. *Microbial Biotechnology*, **5** (6), 738-752.

[16] Cresson, R., Escudie, R., Carrere, H., Delgenes, J.P., Bernet, N., 2007. Influence of hydrodynamic conditions on the start-up of methanogenic inverse turbulent bed reactors. *Water Research*, **41** (3), 603-612.

[17] D'Acunto, B., Frunzo, L., Klapper, I., Mattei, M. R., 2015. Modeling multispecies biofilms including new bacterial species invasion. *Mathematical biosciences*, **259**, 20-26.

[18] Cresson, R., Carrere, H., Delgenes, J.P., Bernet, N., 2006. Biofilm formation during the start-up period of an anaerobic biofilm reactor - impact of nutrient complementation. *Biochemical Engineering Journal*, **30** (1), 55e62.

[19] D'Acunto, B., Esposito, G., Frunzo, L., Mattei, M.R., Pirozzi, F., 2015. Mathematical modeling of heavy metal biosorption in multispecies biofilms. Journal of Environmental Engineering, **142** (9).

[20] D'Acunto, B., Frunzo, L., Mattei, M. R., 2016. Qualitative analysis of the moving boundary problem for a biofilm reactor model. *Journal of Mathematical Analysis and Applications*, **438**, 474-491.