MICROBIAL REDUCTION CONTAMINATION DURING BIOGASIFICATION PROCESS: ESTIMATING THE KINETIC PARAMETERS BY INVERSE PROBLEM.

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Abstract. *The health risk of sludge from wastewater is associated to possible presence of pathogens microorganisms. These microorganisms are derived from human and animal waste contaminated. Many authors have studied the mechanism of microbial inactivation taking into account an isothermal process. Recently, authors are studying microbial inactivation non-isothermal which approaches of the real processes. The objective of this work is to propose the kinetics mechanism for the microbial inactivation, specifically in the process of anaerobic digestion using the sludge sewage. Thus, the posterior treatment in the sludge sewage is very important to eliminate the health risk possibility. The inactivation mechanism was applied to simulate the microbial inactivation by the Arrhenius equation. Using the inverse problem, the Arrhenius parameters were estimated by minimization of least squares norm, Levenberg-Marquardt method, and then validated with experimental data. The model was applied in three microorganisms with different heating rates. Analyzing the results, some restrictions were observed.*

Keywords*: Inverse Problem, Anaerobic Digestion, Levenberg-Marquardt method, Microorganisms inactivation.*

1. INTRODUCTION

The health risk of sludge from wastewater treatment plants is associated to the possible presence of pathogens microorganisms. These microorganisms are derived from human and animal waste, processing in food and biological laboratories responsible for treatment of urban wastewater. These organisms are divided into four categories (EPA 1979): viruses, bacteria, parasites and fungi. The risk of contamination can occur through casual contact or by consumption of contaminated animals, insects, soil and water (EPA 1995). Therefore, to reduce the risk of contamination, some treatments can be applied to the sludge before use or disposal. Among these treatments, both the composting (aerobic digestion) as anaerobic digestion, can be used.

Composting is the simplest treatment, and is based on the decomposition of organic matter in the presence of oxygen. The temperature rises to 55 °C due to the respiration of aerobic mesophilic microorganisms, and the treatment lasts up to 15 days or more (Nazih and Lawrence 2007). However, part of biomass can remains unheated and the pathogens may recover (Vinnerås 2007). Anaerobic digesters produce conditions that encourage the natural breakdown of organic matter by bacteria in the absence of oxygen. This type of conversion provides an effective method for turning residues from livestock farming, food processing, treating agricultural, household and industrial residues and sewage sludge into:

- Biogas (rich in methane) which can be used to generate heat and/or electricity;
- Fiber which can be used as a nutrient-rich soil conditioner; and
- Liquor which can be used as liquid fertilizer.

The digestion process take place in a sealed warmed container (the digester) which creates the ideal conditions for the bacteria to ferment the organic matter. The digestion tank needs to be warmed and mixed thoroughly to create the ideal conditions for the bacteria to convert organic matter into biogas (a mixture of carbon dioxide, methane and small amounts of other gases). There are basically two types of anaerobic digestion process:

- Mesophilic digestion, the digester is heated to 30 – 35 °C and the feedstock remains in the digester typically for 15 - 30 days. This kind of digestion tends to be more robust and tolerant than the thermophilic process, but gas production is less, larger digestion tanks are required and sanitization is performed in a separate process;
- On thermophilic digestion, the digester is heated to 55 °C and the residence time is typically of 12 - 14 days. This kind of systems of methane production is more productive and faster, occur a better inactivation of pathogen and virus, but require more expensive technology, greater energy input and a higher degree of operation and monitoring.

More specifically about the importance of the treatment in the digestion process, it is to control the present pathogens in sewage sludge for protection of humans, animals and plants, providing significant benefits when recycling the sludge, and then returning to the land (Lepeuple et al. 2004). The degree of disinfection is influenced by a variety of interacting operational variables and conditions but it is highly dependent on time and temperature (Pepper and Gerba 2004).

With the aim to determine the required time for a given inactivation process, it is necessary to have information about the heating rate in the mass of sludge inside the digester. The heating rate is measured placing a thermocouple at the center of sewage sludge to record the temperature during anaerobic digestion. In addition, it is necessary to know how the microorganisms are heat resistant in order to define time and temperature for the process.

There are a variety of model for description of microbial reduction. The commonly used is given by (Heldman and Singh 2001) that predicts the survivor population when the initial populations are exposed to elevated temperature during a heating time. This model considers the hypothesis on which every microorganism has its own characteristic heat resistance, defined as the time required to reduce the population by 90% at a constant temperature (Brennan 2006). Thus, this kind of model is knows as isothermal. However, the digestion process does not take place at constant temperature but involves distinct periods. Concerning to the non-isothermal model, some different types are available in the literature (Casolari 1998; Holdsworth and Simpson 2007).

According to (Van Impe et al. 1995), the last few decades there is an interest increased in mathematical modeling and simulation of the different phenomena occurring during thermal processing of foods. Models and simulation techniques are being developed for heat transfer, microbial growth and inactivation, among others. Growth model microbial which depends of temperature was proposed by (Ratkowsky et al. 1982) where an relationship empirical square root between the maximum specific growth rate and the temperature show the growth. (Ratkowsky et al. 1983) expanded their model to describe the growth rate around the optimum and the maximum temperatures.

More realistic model are time-temperature dependent like the ones proposed in many literatures (Zwietering et al. 1991; Van Impe et al. 1995; Valdramidis et al. 2005; Gil et al. 2006; Valdramidis et al. 2006). Three main assumptions, in this kind of models, are in the backstage of the models development, as highlighted by (Valdramidis et al. 2005):(i) no microbial growth occurs during the heating time of the treatment; (ii) there is a limit of temperature below which no inactivation is observed (i.e., k is set to zero for temperatures lower than this limit), and (iii) the historic of the temperature has not a significant effect on the microbial heat resistance. Thus, (Valdramidis et al. 2006) developed an integrated model based in Arrhenius equation, by influence the temperature. The validation was obtained by confrontation of the experiment data about Listeria Monocytogenes. The experiments used were carried out at two heating rates: 16.0 and 90.0 K min^{-1} with temperature ramp from 15 ºC to 90 ºC. As main conclusions the authors acknowledge that the most of the microbial curves have the kinetic of first-order, but this approach should be considered carefully because the vegetative cells do not follow the kinetics inactivation of first-order.

Some authors, in order to confront their models commonly use the so called pseudo-experimental data. This type of approach was already used in studies of kinetics inactivation (Haralampu et al. 1985; Nunes et al. 1991). In (Gil et al. 2006) the pseudo-experimental data for *Listeria Monocytogenes* were generated by the modified Gompertz model (Zwietering et al. 1991) which use heating rates of 18.3 and 110.0 K min^{-1} and final temperature of 60 ºC. They concluded that the heating period greatly affects bacterial inactivation.

In (Corradini et al. 2007) were carried out experiments for *Escherichia Coli* in order of compare the inactivation evolution made by the Weibullian (or power law) model (Peleg and Cole 1998). The main parameters controlled were the heating rates at 1.56, 0.76, 0.52 and 0.15 K min^{-1} and final temperature at 55 ºC. The authors reported that the method apply almost certainly to inactivation that follow kinetics of first order, and remarks that the first order kinetics facilitates the solution because there is the eliminating of one unknown parameter.

Another type of model, re-parameterized Gompertz-inspired model (Gil et al. 2006; Char et al. 2009; Huang 2009), was used in (Miller et al. 2011) and then validated against three experiments for *Listeria Innocua* inactivation. The experiments were made in a liquid medium which is similar to the situation observed in the biogasification of the sludge sewage. The heating rates used for microbial inactivation were 1.50, 1.80 and 2.60 K min^{-1} with final temperature of the 65 ºC. The parameters were estimated for different medium and the authors observed that this change affects greatly the thermal resistance of the bacteria.

For this work, the main objective is obtaining a better understanding of the kinetics of microbial inactivation in biogasification process. For this, the following aims were formulated:

- To formulate a numerical procedure based on the inverse problem algorithm to estimate the Arrhenius parameters of the microbial inactivation;
- Validate the model against some experimental data available in the literature.

2. KINETICS PARAMETERS ESTIMATION

2.1. Inactivation Model

The mathematical model used to describe inactivation of microorganisms is a classical model commonly applied in chemical reactions, where the negative product of an exponential (relationship of Arrhenius equation with the temperature) and a power-law (logarithmical of population) is proportional to inactivation rate. The kind of process does not take place at constant temperature and involves distinct periods. Consequently, the inactivation process is treated like a chemical and an irreversible reaction, Eq. (1).

$$bacteria_{live} \xrightarrow{k} bacteria_{inactived} \tag{1}$$

thus, the rate of inactivation is given by Eq. (2),

$$k(t) = A\exp^{-E/RT(t)} \tag{2}$$

where A is the pre-exponential factor, E is the activation energy, R is the ideal gas constant and T is a defined linear temperature profile. Thus, the inactivation rate for of a reaction of order l is given by Eq. (3):

$$\frac{dN(t)}{dt} = -k(t)[N(t)]^l \tag{3}$$

where N is the logarithmic of the microorganism population in instant t. The initial conditions for the Eq. (3) is $N(0)$ that is the initial population for a given microorganism.

The Eq. 3 is then solved numerically to obtain an expression in function of the Arrhenius parameters. This expression is called the solution of the direct problem, will be detailed next session. This procedure is the initial step for the application of the inverse problem.

2.1. Inverse Problem Methodology

An inverse problem can be defined as the assignment that often occurs in many branches of science and engineering, where the values of some model parameters must be obtained from the measurements data. According to (Dantas et al. 2002) the Inverse problems can be *ill-posed*. The term itself has its origins in the early 20-th century. It was introduced by Hadamard who investigated problems in mathematical physics. According to his beliefs, ill-posed problems did not demonstrates problems real world, but later it appeared how wrong he was. Hadamard defined a linear problem being well posed if it satisfies the following three requirements: (a) existence, (b) uniqueness, and (c) stability. A problem is said to be ill-posed if one or more of these requirements are not satisfied.

Several methods of solution of inverse problems, such as the one used here, involve their reformulation in terms of well-posed minimization problems. By assuming additive, uncorrelated and normally the distributed random errors, with constant standard deviation and zero mean, the solution of the present parameter estimation can be obtained through the minimization of the ordinary least-squares norm (Dantas et al. 2002), Eq. (4).

$$S(P) = [Y - N(P)]^{\mathrm{T}}[Y - N(P)] \tag{4}$$

where $P=[A_g,E_g,l_g]$, with g=1, …, 3, denotes the vector of unknown parameters. The term $[Y\text{-}N(P)]$ is the difference between the measurements and the result of direct problem - the solution of the differential equation Eq. (3). The superscript T above denotes the transpose given by, Eq. (5).

$$[Y - N(P)]^T \equiv \left[\left(\vec{Y}_1 - \vec{N}_1\right),\left(\vec{Y}_2 - \vec{N}_2\right),...,\left(\vec{Y}_n - \vec{N}_n\right)\right] \tag{5}$$

The subscript n refers to the time in which the measurements are taken.

Thus, the inverse problem is solved by using the Levenberg-Marquardt algorithm. This method is quite stable, powerful and straightforward (Özisik and Orlande 2000), and has been used to a diversity of problems. Equation (6) shows its discrete form.

$$\mathbf{P}^{(\kappa+1)} = \mathbf{P}^{(\kappa)} + [(\mathbf{J}^{(\kappa)})^{\mathrm{T}}\mathbf{J}^{(\kappa)} + \mu^{(\kappa)}\mathbf{\Omega}^{(\kappa)}]^{-1}(\mathbf{J}^{(\kappa)})^{\mathrm{T}}[\mathbf{Y} - \mathbf{M}(\mathbf{P}^{(\kappa)})] \tag{6}$$

where $\mathbf{P}$ represents the unknowns parameters vector, the superscript (κ) defines the number of iterations. The parameters μ and $\mathbf{\Omega}$, respectively, are the damping parameter and damping matrix from the method, and it is used to smooth some oscillations and instabilities that can hamper the convergence process making it faster. Finally, $\mathbf{J}$ represents the sensitivity matrix defined by Eq. (7),

$$\mathbf{J} = \left[\frac{\partial \mathbf{N}^T(\mathbf{P})}{\partial \mathbf{P}}\right]^T = \begin{bmatrix} \frac{\partial N_1}{\partial p_1} & \cdots & \frac{\partial N_1}{\partial p_m} \\ \cdot & & \cdot \\ \cdot & & \cdot \\ \cdot & & \cdot \\ \frac{\partial N_N}{\partial p_1} & \cdots & \frac{\partial N_N}{\partial p_m} \end{bmatrix} \tag{7}$$

the elements of the sensitivity matrix are the sensitivity coefficients. They are defined as the first derivative of the microorganism population with respect to each one of the unknown parameters, P_m, where $m = 1,..., M$. Also, they can provide considerable insight to the estimation problem and in the design of the experiment for an optimum accuracy in the estimates. An iterative procedure is required due to the non-linear nature of the estimation problem because the sensitivity coefficients depend on the unknown parameters to be recovered. The iteration continues until the estimation is reached, i.e. when there is negligible change in any component of **J**.

Different versions of the Levenberg–Marquardt method can be found in the literature. The diagonal of the damping matrix $\mathbf{\Omega}^{K}$ and the variation form of the damping parameter μ^{K} used are the one's intrinsic of Mathematica Software.

2.3. Results and discussions

The proceeding of simulation were applied for three different microorganisms: *Listeria Innocua*, *Listeria Monocytogenes* and *Escherichia Coli*. These microorganisms were chosen because measurements data are available in abundance on the literature. For each microorganism the parameters of Arrhenius (*A, E* and *l*) were estimated for only one heating rate and then it were tested for other heating rates to verify its range of applicability.

A set of experimental data were necessary to support the inactivation model. For the *L. Innocua* the heating rate used in the estimation was 1.80 K min^{-1}, for the *L. Monocytogenes* was 90.00 K min^{-1} and for the *E. Coli* was 1.56 K min^{-1}.

In the Fig. 1 are presented the transient behavior of the relative sensitivity coefficients for the three estimated parameters. The values of the parameters used as initial guess and in the sensitivity study, are reported in Table 1.

Table 1. Experimental results for inactivation.

	L. Innocua				***L. Monocytogenes***				***E. Coli***			
Parameters	***N(0)*** (Log cfu mL^{-1})	A_1 (min^{-1})	E_1 (J mol^{-1})	l_1 (-)	***N(0)*** (Log cfu mL^{-1})	A_2 (min^{-1})	E_2 (J mol^{-1})	l_2 (-)	***N(0)*** (Log cfu mL^{-1})	A_3 (min^{-1})	E_3 (J mol^{-1})	l_3 (-)
Values	7.913	$3.33x10^9$	75251.1	1	7.577	5	5000	1	7.913	250	27000	1

In Fig. 1a, the smallest sensitivity coefficient is observed with the pre-exponential factor A_1, which can have major difficulties in its estimation. In Fig. 1b and 1c the same behavior was observed for A_2 and A_3, respectively. Another important aspect that be highlighted is the apparent linear dependence between the parameters A_1 / l_1, A_2 / l_2 and A_3 / l_3, but a careful examination of the different ratios between all the relative coefficients shows that they are not linearly dependent and therefore their simultaneously estimation is feasible.

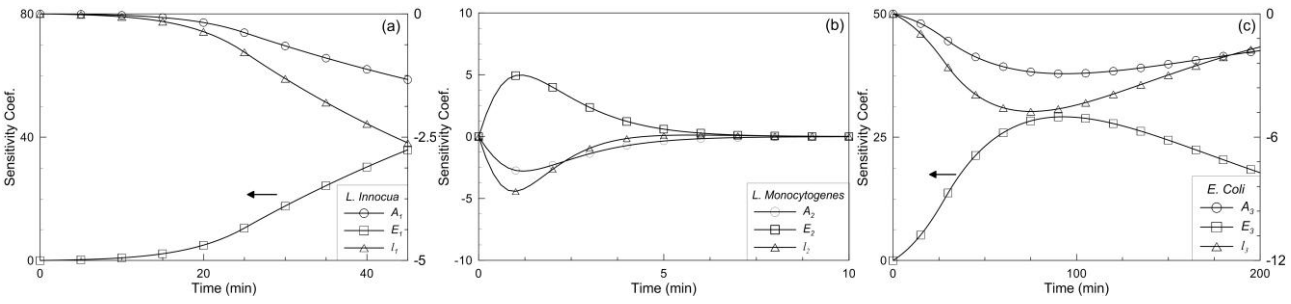

Figure 1. The transient behavior of the sensitivity coefficients.

One can noted by transient behavior of the sensitivity coefficients that a small value of the magnitude of coefficients indicates large changes in the parameters thus the difficult of estimation is bigger (Özisik and Orlande 2000). In all three cases the values of parameters A_j, with j=1, 2 and 3, is smaller (in module), so the complexity of estimation becomes evident when the value of the parameter is analyzed. In the other hand, the values of coefficients sensitivity of E_j, with j=1, 2 and 3 is the bigger between the three parameters and in the analyzing to the errors can be noted a smaller error of estimation.

From the experiments about *L. Innocua* (Miller et al. 2011), *L. Monocytogenes* (Valdramidis et al. 2006) and *E. Coli* (Corradini et al. 2007), the data were extracted and the parameters estimated by minimizing the objective function (Levenberg-Marquardt method), the results are presented in the Fig. 2. The proceeding of simulation were performed at the heating rate of 1.80, 90.0 and 1.56 K min^{-1} respectively. The values of the estimated parameters as well the relative error, for each microorganism, are present in Table 2.

Table 2. Estimated parameters.

	L. Innocua			***L. Monocytogenes***			***E. Coli***		
Parameters	A_1 (min^{-1})	E_1 (J mol^{-1})	l_1 (-)	A_2 (min^{-1})	E_2 (J mol^{-1})	l_2 (-)	A_3 (min^{-1})	E_3 (J mol^{-1})	l_3 (-)
Values	8.808x10^{24}	169876.0	0.997	151.580	17826.9	1.004	1.801x10^{8}	63355.1	0.996
Error (%)	0.193	0.0034	0.183	0.299	0.056	0.113	0.049	0.0018	0.028

For *L. Innocua*, Fig. 2a, the model is in agreement with the experimental data. As the heating rate is slow, the time to reach the temperature of inactivation is large forming a plateau that extends over 20 min. It was observed that the inactivation begins in 323 K and follows the experimental tendency until the final time.

In the Fig. 2b, a higher heating rate at 90.0 K min^{-1} was used to inactivation of *L. Monocytogenes.* The model shows a concordance with the experimental data. In this case the plateau is not present indicating that the temperature of inactivation is quasi-instantaneously reached.

From the Fig. 2c, it was observed the same behavior when compare to the *L. Innocua.* The inactivation temperature was about 305 K. Consequently, it is important to note that the heat resistance of *E. Coli* is lower than the *L. Innocua*.

One can highlight also in Fig. 2a and 2c, when the isothermal temperature is reached the inactivation curves tends to be log-linear as reported by (Aragao et al. 2007; Chen 2007).

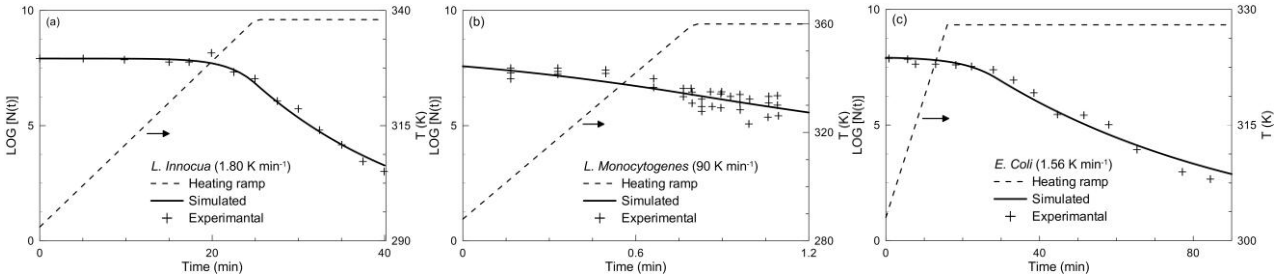

Figure 2. Inactivation evolution curves for three microorganisms with its respective heating rates.

For *L. Innocua* the applicability ranges for the estimated parameters were: close to 1.50 K min^{-1} at the inferior limit and, less than 2.60 K min^{-1} at the superior limit. In the inferior limit the model showed a good agreement with the experiment, Fig. 3a. Still, the model reproduces the classic plateau commented previously, as well, it shows with precision the same temperature of inactivation as observed in the Fig. 2a., being in agreement with the assumptions proposed by (Valdramidis et al. 2005) where with there is a limit of temperature below which no inactivation is observed. On the other hand, it was noted that, since the isothermal temperature in the superior limit is reached, the model moves away of the experimental data, Fig. 3b.

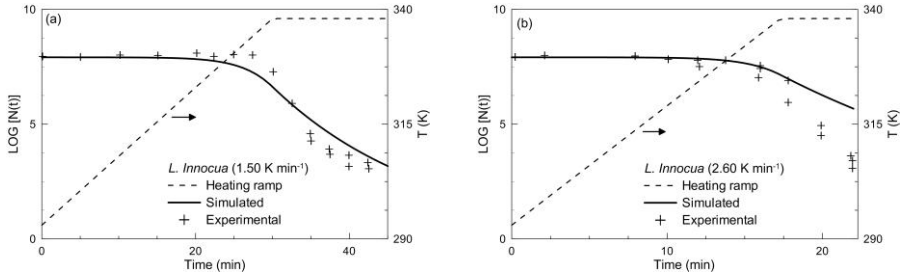

Figure 3. Estimated parameters applied in two different heating rates: Inactivation evolution for *L. Innocua.*

For *L. Monocytogenes* the range of application of the parameters were tested from the inferior limit 16.0 K min^{-1} until the superior limit of 110.0 K min^{-1}. In the Fig. 4c, the parameters estimated describe the inactivation very well. But looking for the Fig. 4a and 4b is clear that the curbs with the parameters estimated distance of experimental data. This is explained by relative difference between the heating rate of estimation and the heating rate of Fig. 4a and 4b.

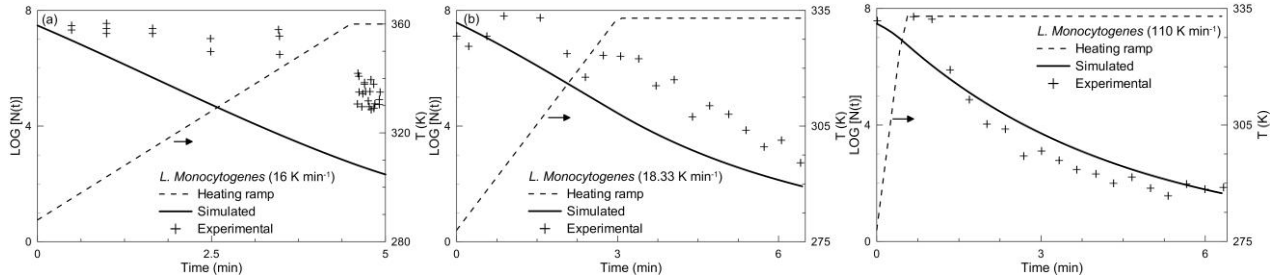

Figure 4. Estimated parameters applied in three different heating rates: Inactivation evolution for *L. Monocytogenes.*

For *E. Coli*, Fig. 5, in all experimental cases tested, the model showed to be efficient in the reproduction of the behavior in terms of shape, inactivation temperature and process time. The applicability ranges for this microorganism extends from 0.15 K min^{-1} to 1.56 K min^{-1}. The superior limit of validity was not tested due to unavailability of experimental data.

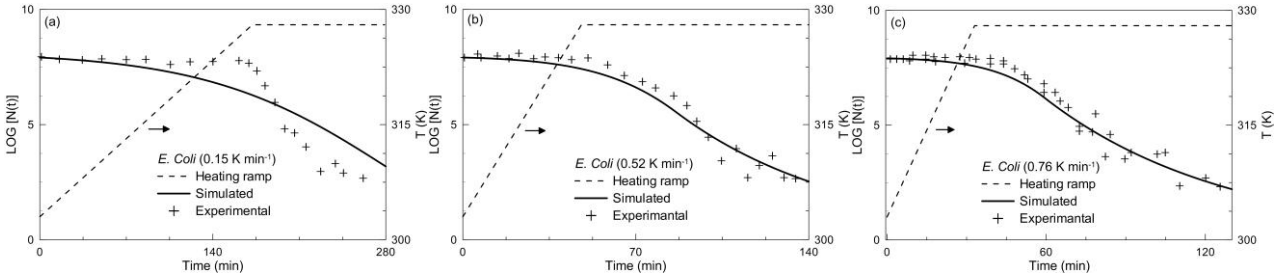

Figure 5. Estimated parameters applied in three different heating rates: Inactivation evolution for *E. Coli.*

3. CONCLUSION

Comparing the temperature profile is evident the influence of heating rate in the plateau and in the holding time. One should be aware that if a slow heating process is chosen, the time of the process should be extended to achieve a specified target of microbial load when compared to a fast heating process.

The model of microbial inactivation can illustrate the two parts in the all process, both in the phase of heating as in the phase isothermal where the inactivation is close of the model log-linear of reduction. It is important underline that the parameters estimated, together with this model, describe process of inactivation in a range of heating rate and not in all heating rate. This limit needs be tested and studies for understand the functioning need be done.

A problem for this procedure is the need of a gradient of temperature bigger than 10 ºC. This problem was perceived with the attempt of simulate the data from (Valdramidis et al. 2008) and the model cannot follow the shape of the experimental data. Some studies admit inactivation of first order, this assumption is validates due to the fact that in the estimation the parameters *l* the values found is close of one.

The medium is an aspect important to, but also the proprieties others, because if any change occurs in the proprieties, the parameters estimated changes also. The results in order of apply to represent the inactivation in the

process of anaerobic digestion are satisfactory and the parameters can be utilized if the parameters were estimated close to heating rate of the anaerobic digestion.

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