

Use of mathematical modeling on the solid-state fermentation processes of fibrous substrates for animal feeding

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The Solid-State Fermentation (SSF) of fibrous residues is one of the most promising techniques for animal feeding production through biotechnological via. In Cuba, from rustic fermentations, several product of great utility have been obtained such as Saccharina, the Sacchamaiz and Sacchaboniato. This study is methodological and presents a general review on the present situation of mathematical modeling of the SSF. The use of this tool to describe the rustic fermentation of fibrous substrates on slid state is included. For that, the equations of balance and energy have been taken into consideration, as well as a logistic equation to describe the microbial growth in these processes. Mathematical modeling in this type of fermentations, allows determining the protein content and estimating the profiles of temperature, height of solid bed, total time of the process and turning time of the substrate to achieve a more efficient process. Up to now, models to predict the performance of rustic SSF have not been reported.

Key words: rustic solid-state fermentation, mathematical modeling, fibrous residues

INTRODUCTION

During the last years, important advances have occurred on the development of Solid-State Fermentations (SSF). Enzymes and ethanol production is among the most important applications of this type of fermentation (Treichel *et al.* 2010) (Wang *et al.* 2010). These fermentation processes allow increasing the protein content and diminish the fiber of some agricultural products to obtain new options as animal feeding (Rodríguez *et al.* 1998). In the case of this application, the SSF are carried out under rustic conditions as the whole process occurs on cement floor, where the main variables of the process cannot be controlled.

Some products of this type have been developed at the Institute of Animal Science (ICA), such as Saccharina, Sacchamaiz, Sacchasorgo, the fermentation of sugar cane with cattle manure and Sacchaboniato. From these products, several studies have been conducted at laboratory and higher scales.

The objective of the laboratory studies is to improve the nutritive value of the product and they are carried out in Roux flasks (Ramos *et al.* 2006 and Ramos *et al.* 2007). The researches on the floor are focused on studies on the height of the solid beds (Rodríguez *et al.* 2006 and Carrasco *et al.* 1997), the turning of the substrate (Carrasco *et al.* 1997) and the fermentation time (Lezcano and Marti 1997). The rustic SSF are not only applied at the ICA, but they are also used in cattle production units from the Agriculture and Sugar Cane Ministers (Lezcano *et al.* 1994).

In the case of the controlled SSF, the design of the fermentation chambers has been a great advance on studying the different kinetic parameters and those of

transportation during the fermentative process. However, the results with this technique have not been successful, so their characterization has been needed to improve the design and the operation strategies (Dustet 1999). In this sense, new equipments have been designed, such as bioreactors, which are not applied in the fermentations under rustic conditions, due to their high costs and the low added value of the products obtained through this via.

Together with the bioreactors design, the mathematical modeling of this type of process was also developed. As consequence, the rustic SSF were excluded due to their low application.

The development of models to describe the biological and transportation phenomena during the fermentation process improved the comprehension of many SSF aspects (Pandey 2003): the biochemistry, the process engineering, (Raghavarao *et al.* 2003), the design and operation and scale of bioreactors (Mitchell *et al.* 2003). In the case of rustic SSF, the mathematical modeling is a very important tool for conducting the laboratory studies. It will allow estimating the fermentation time, the maximum bed height and the turning time of the substrate to keep temperature in a proper interval for growth, the desired protein level on the product and the optimization of the operation conditions. An important diminishing on the number of experiments has been achieved in all cases. The objective of this study was the use of the mathematical modeling on the SSF of fibrous substrates for controlled and rustic conditions, apart from proposing a mathematical modeling to describe this last process.

MATHEMATICAL MODELING ON SSF

The concept of modeling on SSF refers to the search of mathematical expressions that would represent the system under study. The importance of the models used is that they allow obtaining the values of parameters that explain a particular process and offer an idea of how the different fermentation phenomena are combined to control the global yield. Models are, besides, the basis for assessing the process and they are very important for designing the equipment and control criteria (Singhania *et al.* 2009).

The models can be divided into two main groups: kinetics and of mass and energy. Those of the first group consider the growth kinetics of microorganisms and their affectations due to the environmental conditions. The second group considers the mass and energy preservation laws in the substrate bed and of their interaction with the system (Mitchell *et al.* 2002).

Generally, the models of mass and energy balance are obtained from the equations of transportation phenomena, which include the movement of a property towards several directions. For example, for preserving

the energy, the equation is as follows:

$$\rho C_p \left(\frac{\partial T}{\partial t} + v_x \frac{\partial T}{\partial x} + v_y \frac{\partial T}{\partial y} + v_z \frac{\partial T}{\partial z} \right) = k \left(\frac{\partial^2 T}{\partial x^2} + \frac{\partial^2 T}{\partial y^2} + \frac{\partial^2 T}{\partial z^2} \right) + S$$

The first term represents the temperature variation due to the convection. The second, the temperature changes due to the conduction mechanism. The term is the heat source per volume unit (Garcell *et al.* 1984). Some terms may be canceled in this equation, depending on the study. For air-forced bioreactors, for example, the conductive term is despicable in respect to the convective. This is because the metabolic heat released during the fermentation is extracted through the air flow. The diffusive mechanisms prevail in the rustic SSF. Then, the convective term can be despised and the heat transportation by conduction predominates. The same occurs with the general equation of transport phenomena for mass conservation. Therefore, the models existing for bioreactors have no application on the processes conducted under rustic conditions.

KINETICS MODELS

The different kinetic models for the SSF are: linear, exponential, logistic (more used) and of rapid acceleration/slow des-acceleration (Mitchell *et al.* 2004). Table 1 shows the differential equations for each of them.

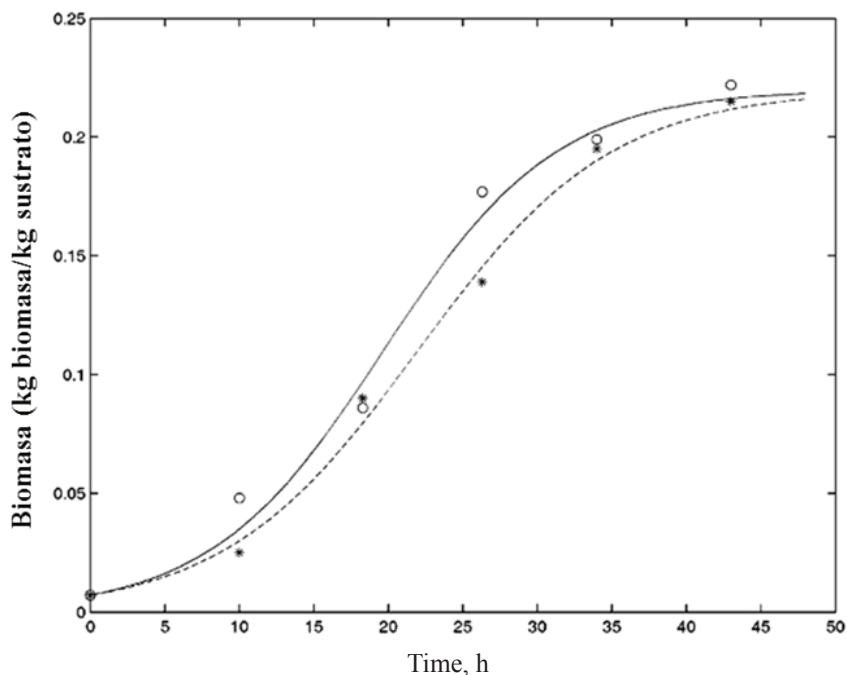
Ali and Mahmoodzadeh (2009) propose a model for the SSF of wheat bran with *Aspergillus niger*, including the logistic equation for describing the growth of this microorganism. The results of the kinetic model were compared with experimental data reported by Hamidi-Esfahani *et al.* (2004) and they are shown in figure 1, showing that the model can predict the experimental results. Applying this model, time and resources are saved. Besides, the needed information of the process under study is obtained.

Among the most recent studies, that conducted by Hashemi *et al.* (2011) studies the modeling of the different stages of the growth curve of *Bacillus sp.* KR-8104 in wheat bran, as well as the α -amylase production from this fermentative process. The experiments are developed with an exponential model to predict microbial growth and they were based on the variations of the substrate dry weight.

The description of the environmental conditions (temperature, pH, among others) is another important aspect to be considered in the SSF modeling. The models obtained allow predicting these conditions, as a time function, as well as the response of the microorganisms to them (Mitchell *et al.* 2004).

Table 1. Growth differential equations in the SSF processes

	Differential way
Linear	$\frac{dX}{dt} = k$
Exponential	$\frac{dX}{dt} = \mu X$
Logistic	$\frac{dX}{dt} = \mu X \left(1 - \frac{X}{X_m} \right)$
Two phases	$\frac{dX}{dt} = \mu X, t < t_a; \frac{dX}{dt} = [\mu L e^{-b(t-t_a)}] X, t \geq t_a$



(○) Experimental data and (—) Model; (*) Experimental data and (- -) Model

Figure 1. Comparison between the model and the experimental data of Hamidi-Esfahani *et al.* (2004)

MODELS OF MASS AND ENERGY BALANCE

The models of mass and energy balance in a bioreactor describe the transference of mass and heat in the solid substrate bed and the interchanges between this phase and others. Generally, the majority of models focus on the energy balance (Sahir *et al.* 2007), although some include the oxygen transference (Rajagopalan and Modak 1995) and others, the water balance (Weber *et al.* 2002).

Among the models referred in the literature, that proposed by Sangsurasak and Mitchell (1998) includes the evaporation and transference of heat in vertical directions and radial in cylindrical geometry in a packed-bed bioreactor. When comparing the results of this model with the experimental data reported by Ghildyal *et al.* (1994), it was concluded that the model of Sangsurasak and Mitchell (1998) can reproduce these results (figure 2).

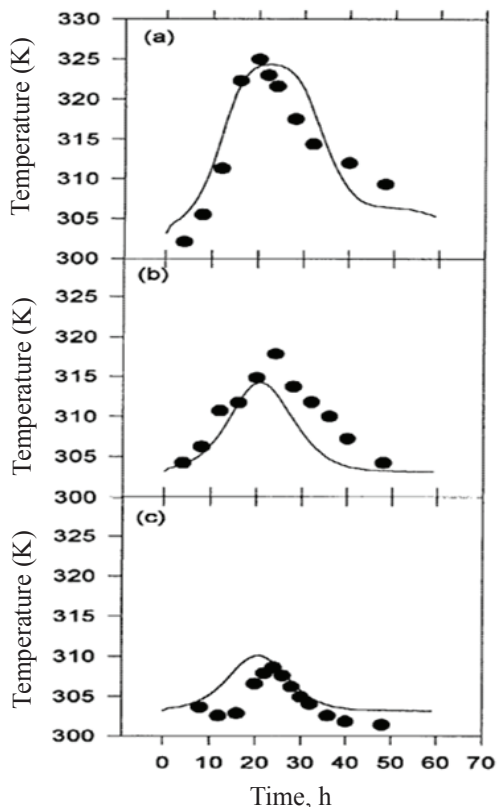
The model of Weber *et al.* (1999), apart from the energy balance, includes that of water, allowing describing the performance of the culture humidity during fermentation. Dustet and Izquierdo (2004) propose a modification to this model, so it is expressed in function of the growth kinetics of microorganisms. The substrate of this study was sugar cane bagasse and the microorganism was the cellulolytic fungus *Aspergillus niger*. The mathematical modeling to describe the SSF process includes the balances of mass and energy, kinetic and auxiliary equations.

The validation criterion adopted in this study was the comparison of the temperature of the air flow,

measured experimentally and that predicted by the model. The results of Dustet and Izquierdo (2004) are reported in table 2, showing that the values obtained from the model do not differ from those of the experiments.

The model of Ali and Mahmoodzadeh (2009) is among the most recent models of energy balance. It includes the terms generation of metabolic heat, convection and water vaporization. The results of this model are shown in figure 3, proving that they are similar to the experimental data reported by Ghildyal *et al.* (1994). This indicates that it can describe the model under study.

Up to the present, all the models proposed for SSF bioreactors cannot be applied under rustic conditions, due to the differences between their dynamics. For those SSF conducted on the floor, the control of the variables is more difficult as the process is exposed to the environmental conditions. Besides, it has to be considered that many times a mixed microbial community is present, differentiating also this type of fermentation from those controlled, that use pure cultures. This shows that there are differences in the physiological performance of the microorganisms, so it is harder to control and keep the best conditions for growth. In this sense, Kreger (1984) conducted studies with pure cultures and found that there is an optimum temperature for each type of microorganism. However, it is harder to find it and keep it in mixed cultures.

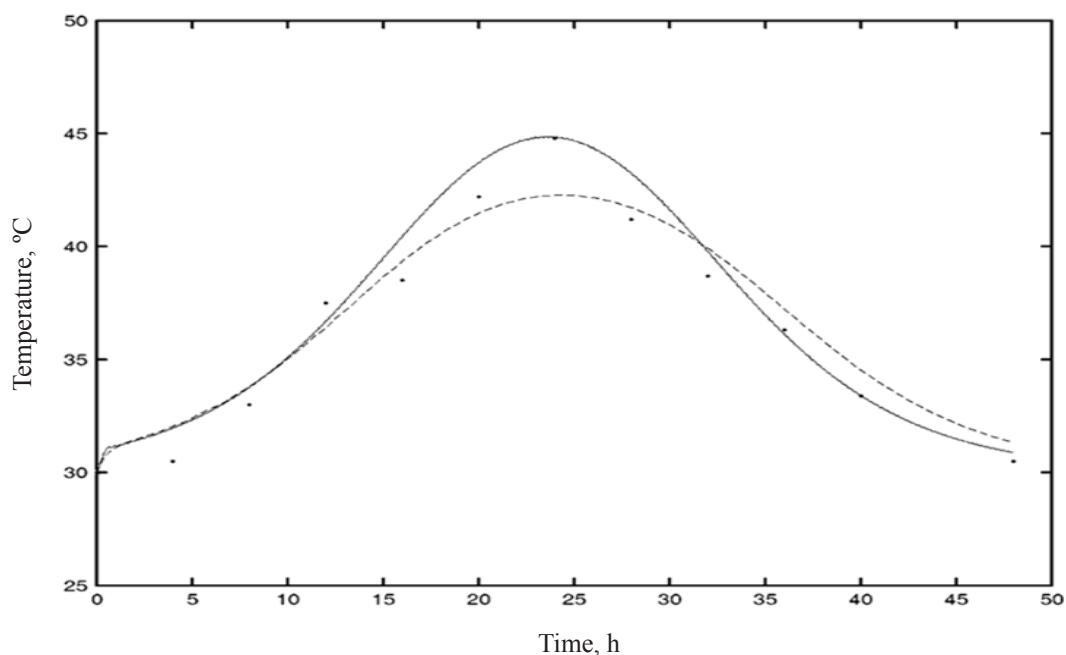


The results are shown for different superficial rates (a) 0.0047 m/s, (b) 0.0141 m/s and (c) 0.0235 m/s.

Figure 2. Comparison of the model results (line) with the experimental data reported by Ghildyal *et al.* (1994) (symbol)

Table 2. Temperature of the air flow for the SSF with *Aspergillus niger*

Time interval, h	Experimental temperature, K	Theoretical temperature, K	Relative error, %
0-10	309	313.8	1.60
11-20	308	310.5	0.80
20-48	308	311.0	0.97



(..) Experimental data; (--) (—) Models

Figure 3. Comparison of two heat transference models with the experimental data of Ghildyal *et al.* (1994)

The convective term predominates in the mathematical modeling for processes in forced-air bioreactors. This causes that the transportation processes occur at a higher speed than through a diffusion mechanism. This, among other advantages, increases the height of the solid bed and, therefore, allows working in smaller spaces.

An important aspect to be considered is that rustic

fermentation may be a productive variant for small communities. In this case, allows the self-supply of animal feeds, without great investments, required for the fermentations in bioreactors. Besides, this type of feed does not have an added value, as the obtainment processes are not complex in equipment and procedures.

MATHEMATICAL MODELING OF THE RUSTIC SOLID STATE FERMENTATIONS

One of the biggest problems of rustic SSF is that the parameters leading the process cannot be easily controlled. Among the troubles of this fermentation are the high temperature gradients, due to the

accumulation of metabolic heat, as forced air is not used during the process. So far, studies on modeling this type fermentation to deep in it and improve its development.

CHARACTERISTICS OF THE SYSTEM FOR RUSTIC FERMENTATIONS

The work system for rustic fermentations is solid bed extended on cement floor, assuming that the geometry is rectangular (figure 4) and the ratios height/length and height/width are small. The system is heterogeneous

(four phases: solid, liquid, gas and cellular) and pore like. It is exposed to environmental conditions and the work is in non-stationary estate.

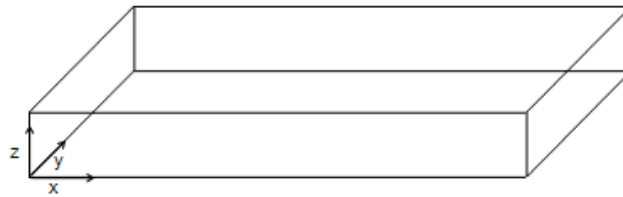


Figure 4. Scheme of the work system

EQUATIONS FOR THE MATHEMATICAL MODELING OF RUSTIC FERMENTATIONS

a) Kinetic equation. The logistic equation is used to describe the growth kinetics of microorganisms, equally to the controlled fermentations.

$$X = \frac{X_m}{1 + \left[\left(\frac{X_m}{X_0} \right) - 1 \right] e^{-\mu t}}$$

Where:

X is the biomass concentration (kg of biomass/kg of substrate)

X_m and X_0 represent the maximum and initial concentration of biomass, respectively

(kg of biomass/kg of substrate)

μ is the specific growth rate (h^{-1})

t is the time (h).

The logistic equation is one of the most used, as it gives a proper approach of the whole growth curve, where the delay phase and the growth ceasing in the last fermentation stages are included in only one equation (Mitchell and van Meien 2000). It also allows describing the growth of any microorganism as those of mixed populations. The biomass content derived from the fermentation can also be determined, which is equivalent to one percent of proteins and is represented by the variable .

ENERGY BALANCE

The energy balance for the process is expressed as follows:

$$\rho_b C_{pb} \frac{\partial T}{\partial t} = k_b \left(\frac{\partial^2 T}{\partial x^2} + \frac{\partial^2 T}{\partial z^2} \right) + \frac{\Delta H_0 \rho_s (1 - \varepsilon) dX}{Y_{x/o_2}} \frac{dX}{dt}$$

Where:

X is the biomass concentration (kg of biomass/kg

of substrate)

t is the time (h)

T is the temperature ($^{\circ}C$)

C_{pb} is the heat capacity of the solids bed (kJ/kg $^{\circ}C$)

K_b is the heat capacity of the bed (kJ/mh $^{\circ}C$)

ρ_b is the density of the solids bed (kg/m³)

ρ_s is the substrate density (kg/m³)

ε is the porosity
 x and z represents the length and height of the solids bed, respectively (m)

ΔH_o is the standard combustion heat of organic molecules (J/kg of oxygen)

X_{x/O_2} is the yield coefficient biomass/oxygen (kg of biomass/kg of oxygen).

This equation determines the temperature profile in the solids bed. The first term represents the temperature variation in respect to time; the second, the conductive term in two of the three space directions; the third represents the generation of metabolic heat during the process.

MASS BALANCES

The equations that allow determining the rates of the substrate and oxygen intake are also considered, as well as the water production rate, obtained from the fermentative process. The balances are only presented for the solid stage.

- Substrate:

$$(1 - \varepsilon) \frac{dC_s}{dt} = - \frac{\rho_s}{Y_{x/s}} \frac{dX}{dt}$$

- Oxygen:

$$\varepsilon \frac{dC_{O_2}}{dt} = - \frac{\rho_s}{Y_{x/O_2}} \frac{dX}{dt}$$

- Water:

$$(1 - \varepsilon) \frac{dC_{H_2O}}{dt} = \frac{\rho_s}{Y_{x/H_2O}} \frac{dX}{dt}$$

Where:

X is the biomass concentration (kg of biomass/kg of substrate)

t is the time (h)

C_s , C_{O_2} and C_{H_2O} is the concentration of solid, oxygen and water, respectively (kg/m³)

ρ_s is the substrate density (kg/m³)

ε is the porosity of the bed of solid

$Y_{x/s}$ is the yield coefficient biomass/substrate (kg of biomass/kg of substrate)

Y_{x/O_2} is the yield coefficient biomass/oxygen (kg of biomass/kg of oxygen)

Y_{x/H_2O} is the yield coefficient biomass/water (kg of biomass/kg of water)

The mass balances allow estimating the intake rate of substrate and oxygen, as well as water production rate. In the case substrate, it is of great importance because there is a great intake of this compound during fermentation. Besides, the metabolic heat is generated, mainly, from the substrate that is energy source. The oxygen balance should also be considered as it is an important nutrient for aerobic fermentations. Also, because there is a relation between the amount of heat released and the oxygen consumed in the process.

The function of water in the SSF is multiple

The conduction term is only considered in the energy balance, as the fluid in contact with the system is the environmental air. Besides, the transportation mechanisms manifested in this type of process are purely diffusive, different from those processes occurring in the air-forced bioreactors which are convective mechanisms.

The energy balance allows predicting, in time and position, the system temperature, being of great utility as it is a variable that affects the microbial growth during fermentation. The temperature profiles are directly related with the kinetics, as the cell population increases, the metabolic heat develops.

and it is an indispensable component in biomass composition. Another important aspect is that, as the energy transportation is by diffusion, there is then an energy interchange with the environmental air, with a determined temperature and humidity, so the vaporization takes place. The water is also a vehicle for enzymes and nutrients as it facilitates the gas interchanges. A high humidity in the substrate causes low oxygen diffusion and high pollution. On the contrary, low humidity provokes the limited growth of microorganisms and diminishes the substrate availability (Mitchell *et al.* 2002). For these reasons, the mass balance for this compound has to be considered in the mathematical modeling of the SSF.

The mass and energy balances are related through the yields biomass/substrate and biomass/oxygen, the amount of the species pointed out, obtained or consumed in the fermentative process. The yields depend on the microorganism and the substrate, as well as on the growth conditions. They also allow relating the kinetics and thermodynamics of the process.

In general, the balance equations for rustic SSF would allow estimating the temperature profiles, determining the protein content, the total time of the process and that of the turning of the substrate, as well as the best bed height in order to achieve a good fermentative process. This last is one of the most important factors to be controlled, as the solids bed width is higher, higher temperatures would be found, mainly due to the high capacity of thermal isolation of the substrate, which impedes the dissipation of the metabolic heat generated during fermentation. The high temperature gradients limit growth and, even, kill the microorganisms (Mitchell *et al.* 2002).

Rodríguez *et al.* (2006) conducted studies on the rustic SSF of sugar cane with sweet potato (*Ipomea batata* Lam), for 10 and 15 cm of height of the solids bed. These authors demonstrated that, for 10 cm, temperature kept below 40°C, optimum value for a great part of the environmental microorganisms. Likewise, they obtained better results for the rest of the fermentative indicators,

as well as higher microbial growth and protein synthesis.

Temperature may vary considerable during the fermentation and, these variations, influence on the growth and formation of the product. For this reason, it is convenient to include the effects of this variable on the kinetic growth models. One of the most used is that stated by Saucedo-Castañeda *et al.* (1990) for the specific growth rate, in function of temperature:

$$\mu = \frac{A e^{\left(\frac{-E_{a1}}{R(T+273)}\right)}}{1 + B e^{\left(\frac{-E_{a2}}{R(T+273)}\right)}}$$

FINAL CONSIDERATIONS

The mathematical modeling of rustic SSF may offer greater information of this process, as in this type of fermentation; the variable control is more difficult. Besides, the modeling allows knowing the fermentation time to reach a concentration level of the biomass, as well as the level of true protein of the product under certain environmental conditions: environmental temperature, humidity and air rate. It also makes possible to know the maximum height of the solids bed in order to achieve a homogeneous product in biomass concentration and estimate the temperature profiles. The height is in

Where:

μ is the specific growth rate (h^{-1})

T is temperature ($^{\circ}\text{C}$)

A is an empiric constant (h^{-1})

B is an empiric constant (-)

E_{a1} and E_{a2} are activation energies (J/g mol)

R is the universal constant of gasses ($\text{J/g mol}^{\circ}\text{C}$)

function of the growth kinetics and the heat evolution for the cell population used. That is, it won't be a fixed value for all the possible products that may be obtained. The mathematical modeling in this type of fermentation is a useful tool that allows determining the total time of the process and when to turn the substrate to achieve a more efficient process. Up to date, no models to predict the performance of the rustic SSF have been reported. Those existing for bioreactors are not applied for these fermentations due to the dynamic differences between both processes.

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