INDIVIDUAL-BASED MODELLING AND SIMULATION OF MICROBIAL PROCESSES: YEAST FERMENTATION AND MULTI-SPECIES COMPOSTING

C. Prats, J. Ferrer, A. Gras, M. Ginovart
Universitat Politècnica de Catalunya, Barcelona, Spain

Corresponding Author: C. Prats, Dept. Física i Enginyeria Nuclear, Escola Superior d'Agricultura de Barcelona, Universitat Politècnica de Catalunya
Avda. Canal Olímpic 15, 08860 Castelldefels (Barcelona), Spain; clara.prats@upc.edu

Abstract. Controlled microbial activity is the core of many industrial processes that require efficient, cheap and clean bio-chemical transformation of input products. Two examples are yeast fermentation and composting process carried out by multi-species microbial ecosystems. Both processes have been widely studied and modelled in order to better understand, predict and control the evolution of industrial systems from a given initial state. Most of the existing models are population based continuous approaches (top-down). An alternative approach is Individual-based Modelling (bottom-up). INDISIM is a discrete and spatially explicit Individual-based Model. It sets the rules that govern each microbe and its interaction with its local environment and neighbouring microorganisms. The physical space is divided into spatial cells, and the environmental processes are also locally defined. Then it performs simulations including a large number of microbes, and the behaviour of the whole system emerges as a result of these simulations. In this study, we present some examples of specific contributions of INDISIM to the modelling of fermentations and composting processes with special attention to the challenges faced by continuous models in dealing with (i) microbial activity, (ii) environmental conditions and processes, and (iii) external manipulation.

1 Introduction

Controlled microbial activity is the core of many industrial processes that require efficient, cheap and clean bio-chemical transformations of input products. Two examples of industrial applications that are increasingly being studied in order to improve their efficiency are yeast fermentation and multi-species composting processes. The improvement of processing methods often requires the scaffolding provided by quantitative models.

Modelling of fermentation and composting processes involves the description of both the microbial kinetics and the bioreactor performance. Such models face some sticky issues, such as dealing with microbial heterogeneity and different metabolic reactions occurring inside the microorganisms, and also covering the diversity of processes that take place in the environment in different spatial and temporal scales. Furthermore, observations made in the laboratory are not easily extrapolated to industrial systems because the processing may be very sensitive to environmental and performance conditions. All these factors suggest that these kinds of processes are explained by coarse reduction only with difficulty.

A complete model of such processes would entail the description of each volume element in the bioreactor, together with the monitoring of every chemical reaction of each individual cell in the population. This is practically impossible, and probably also worthless. Therefore, simplifications must be introduced, e.g., assuming no concentration gradients throughout the bioreactor, simplifying the individual metabolic networks or considering the population as a whole and all the individual cells acting similarly. The degree of detail to be included in a model depends on the model application and the questions addressed. All in all, the study of microbial processing applications requires a balanced description of both the bioreactor and the microbial community.

The mechanistic modelling of microbial activity through an individual-based approach has proved to be a powerful tool to deal with this kind of system. Such an approach is based on the description of the behaviour of each microbe. It accounts for the cellular morphology and metabolism, and considers both cell variability among the population and uncertainty about individual behaviour. It models bioreactor performance by describing the external flow patterns and the mass transfer processes inside the reactor.

1.1 Mathematical modelling of yeast fermentations

Ethanol fermentation is the biological process in which organic matter is converted by microorganisms into simpler compounds, such as sugars, which are further fermented to produce ethanol and CO_2. There is a great deal of information on fermentation processing available in the literature, including specific reports and reviews [14]. Many bacteria, yeasts and fungi have been reported to be successfully employed to produce ethanol, although historically the yeast *Sacchaomyces cerevisiae* is the most used species. This yeast can grow on simple

sugars such as glucose, and also on disaccharide sucrose. We will restrict ourselves to the former process in this study.

Additionally, the asexual reproduction of yeast facilitates reasonably fast growth, and it is normally used in industrial processes. There are two means of vegetative reproduction: through the production of buds and through the formation of cross-walls in single cells. We are concerned here with budding reproduction [19].

Existing models to study microbial activity can be classified into different categories. Structured models account for various chemical components and their interactions within the cell. By contrast unstructured models are based on the simplifying assumption that detailed modelling of intracellular behaviour is not essential to describe cell growth. Segregated models account for differences between individual cells in terms of properties such as cell mass, size or age. Unsegregated models are based on the simplifying assumption that individual cells have identical physical and chemical properties [21]. Unstructured and/or unsegregated models are easier to handle and analyze but give a poor description of transient operating conditions. Control models for routine operation of industrial fermentations are often based on simple unstructured and/or unsegregated models. Nevertheless, yeast populations are typically heterogeneous with respect to individual cell properties (e.g. biomass composition, genealogical age, cell size, vitality, et al.). Therefore if a better description is required, segregated and structured models are built by introducing more intracellular variables and/or characteristics.

Various kinetic models have been proposed in the literature for freely suspended yeast cells in either batch or continuous operation [14, 15]. These models have been used to predict the influence of well-defined operating parameters on the measurable outcome of the processing system: e.g., cell concentration, substrate utilization rate, and ethanol production rate. However, many issues can not be tackled through kinetic models. For instance, although four hindrances (substrate limitation, substrate inhibition, product inhibition, and cell death) are known to affect ethanol fermentation, none of the models reviewed by Lin and Tanaka [14] accounts for these kinetic factors simultaneously.

We can divide the large number of reactions occurring during yeast growth and ethanol formation into three types: i) substrate uptake, ii) intracellular reactions, i.e., conversion of intracellular substrates into biomass components and metabolic products, and iii) product excretion, i.e., transport of the metabolic products across the cytoplasmic membrane. Many models use the Monod equation in a modified form in order to take into account the inhibiting effect of the end product, the substrate or the self-inhibition. Equations proposed to model the inhibiting effects on yeast growth and population dynamics can be linear, exponential, or hyperbolic. They may consider a critical concentration of the inhibitors above which cell growth is impeded, or other kinds of influence. There are also far more descriptions of product formation velocity by means of equations similar to those that describe cellular growth. The most general equation describing the kinetics of substrate consumption takes into account the consumption of substrate in the formation and maintenance of biomass and the formation of ethanol. However, the various equations found in the bibliography have been proven to be contradictory [15].

1.2 Mathematical modelling of composting process

The composting process has been defined as ‘the biological decomposition and stabilization of organic substrates under such conditions that allow the development of thermophilic temperatures as a result of biologically produced heat, with a final product sufficiently stable for storage and application to land and without adverse environmental effects’ [9]. Such a long and detailed definition suggests that we are dealing with a complex process, in which many agents (microorganisms) are involved and which must occur under very controlled conditions.

Organic matter transformation processes by microbial activity are of great importance in the waste treatment industry, since present day society produces a huge amount of organic wastes that must be treated. In particular, the composting process gives an added value to these wastes because the resulting product can be reused as fertilizer. For this reason, the composting process has been studied for many years with both practical and theoretical approaches.

Mathematical models of the composting process have appeared in the literature since 1976 [16,10]. Most of them are based on the solution of coupled heat and mass balance equations in time from a macroscopic point of view [12], although the number of models that start from the microbial activity is increasing [13,18]. Usually, they are lumped parameter models; that is, they consider the reactor content as a whole and set the global balance equations [13], although some of them may explicitly distinguish among solid, liquid and gaseous phases [18]. Several authors have introduced spatial heterogeneity in their models, in order to take into account different spatially distributed factors [2].

None of these models can predict the evolution of all the involved variables simultaneously (temperature, moisture content, oxygen concentration, CO_2 production, microbial biomass, substrate composition and water vapour, among others) [16]. Moreover, a composting model should be versatile enough to reproduce the enormous pool of possible states that occur in a real composting pile. This would be possible with a model that encompassed the process from the microscopic microbial activity to the macroscopic ventilation processes. It

would need to cover the mass transfers among the different compounds and among the three phases, and also make possible the study of spatial heterogeneity.

1.3 Individual-based Models and INDISIM

Both of the above-mentioned processes, fermentation and composting, have been widely studied and modelled in order to better understand, predict and control the evolution of industrial systems from a given initial state. Most of the existing models are population-based continuous approaches (top-down). They describe the population dynamics and the changes in the concentration of relevant extracellular compounds using differential equations. As has been shown, this mathematical modelling approach to fermentations and composting processes provides a wide pool of useful and interesting results, but is limited by the inherent complexity of the systems under study.

An alternative approach is Individual-based Modelling (bottom-up). Continuous models and Individual-based Models (IbMs) are not exclusive but rather complementary methodologies: both have strengths and weaknesses that can be profited and which counterbalance each other [3].

INDISIM is a discrete and spatially explicit IbM. First, the rules that govern each microbe and its interaction with its local environment and neighbouring cells are set. The physical space is divided into spatial cells, and the environmental processes are also locally defined. Then, this model is implemented in an INDISIM simulator. Simulations including a large number of microbes are performed, and the behaviour of the whole system emerges as a result. INDISIM was initially developed to study bacterial cultures [4], and it has shown to be versatile and useful to deal with other kinds of microorganisms. INDISIM-YEAST [6,7] and INDISIM-COMP [9] are two applications focused on the study of yeast fermentations and composting processes, respectively.

INDISIM allows individual differentiation with regards to microbial biomass morphology, composition and functionality. It is also spatially explicit, so it allows modelling of the environmental non-isotropic processes that may produce spatial heterogeneity. Moreover, the condition of IbM facilitates the simulation of external manipulations, either continuous, discrete or punctual. It is a versatile tool that, once built, can be applied to the study of different configurations and operating regimes of the bioreactor with slight modifications.

In this study, we present some examples of INDISIM's specific contributions to the modelling of fermentations and composting processes. In particular, we pay special attention to how it deals with the challenges faced by continuous models in dealing with (i) microbial activity, (ii) environmental conditions and processes and (iii) external manipulations. Different aspects of this modelling exercise are discussed in the context of our methodology and some preliminary simulation results are presented.

2 INDISIM-YEAST

A detailed description of the simulator can be found in previous works [6,7]. Some key ideas to connect with the simulation results are presented below.

2.1 Modelling the medium

We assume that yeast grows in the bulk of a liquid medium. It is locally described by variables that are space and time dependent. They control the amount of the abiotic components glucose (the nutrient particles) and ethanol (the end product particles) relevant to yeast cellular activity. The spatial simulation domain is a 10x10x10 three-dimensional grid composed of cubic cells in which the number of yeast cells, glucose and ethanol particles is controlled at each time step. The extracellular concentration of substances varies as glucose particles are consumed and ethanol is excreted by the yeast cells. Some manipulation tasks on the system can be performed: i) for a closed system – e.g., a batch culture – with no entry or exit of individual yeast cells and/or substrate particles from the outside, stirring is represented as the homogeneous redistribution of particles; ii) for an open system – e.g., a fed-batch or a continuous culture – external fluxes are represented as the entry and/or exit of individual yeast cells and/or substrate particles into/out of the space domain. The use of immobilized yeast cell systems will be investigated in the near future.

2.2 Modelling a single yeast cell

At each time step, each single organism (individual yeast cell) is defined by a set of parameters and time-dependent variables that represent relevant properties: its position in the spatial domain; its biomass (volume or size assuming spherical shape); its genealogical age as a number of bud scars on the cellular membrane; the reproduction phase in the cellular cycle where it is, namely *Phase 1* – the unbudded and *Phase 2* – the budding phase; its "start mass", i.e., the mass needed to change from the unbudded to the budding phase; the minimum increase in biomass for the budding phase; the minimum time required to complete the budding phase; and its survival time without satisfying its metabolic requirements. Some of these are fixed when the yeast cell is set, and others are modified at each time step. Randomnesss is introduced when setting their initial values in order to represent individual variability.

Additionally, every microorganism must follow a set of rules that account for its activity: motion; uptake of glucose and excretion of ethanol; cellular maintenance; production of new biomass; budding reproduction; cell viability; and death. Randomnesss is introduced when the rules are applied to represent uncertainty in the individual course.

- *Uptake:* the simulator takes into account that the nutrient uptake is affected by the number of bud scars, since they reduce the cellular membrane's effective surface. For the moment, it only considers glucose as nutrient and ignores the consumption of ethanol by the cells, and consequently diauxie is never presented. Other substances, such as nitrogen, oxygen, carbon dioxide and ammonium have been considered to be non-limiting in this study and are not introduced in the simulator yet.
- *Reproduction:* during the unbudded phase the yeast cell gets ready for budding. It enters the budding phase once it has attained a minimum cellular mass, the "start mass", and a minimum increase in biomass. The budding phase lasts until two conditions are fulfilled: a minimum time interval must go by to allow the DNA replication and a minimum amount of biomass must be created.
- *Viability:* the factors that may limit nutrient uptake are (i) ethanol excess; (ii) low glucose concentration; (iii) insufficient surface-to-volume ratio; and (iv) excessive number of bud scars (genealogical age). A deficient nutrient uptake may not satisfy the energy requirements for cellular maintenance. The viability of each individual yeast cell is checked at each time step, and unviable cells are removed from the list of active cells.

2.3 Some simulation results

Some simulation results are presented below with the objective of illustrating the possibilities opened up by INDISIM-YEAST. They should be taken as preliminary results of topics to be further investigated in depth.

INDISIM-YEAST was been initially applied to model and simulate the behaviour of a stirred batch culture (no entry/exit of culturing broth or yeast cells). Therefore, the relation between the cellular activity, the population and the concentration of substrate/product is straightforward. The closed system allows limitation due to product excess inhibition or to nutrient scarcity. Stirring the culture allows not having to consider local diffusion limitations.

Microbial activity. Some kinetic models represent the evolution of such cultures making an explicit reference to different population growth phases: lag phase, exponential growth phase, stationary phase and mortality phase. They may use different sets of equations to describe the system, one for each of the growth phases. The rules governing the individuals stated by INDISIM-YEAST remain the same throughout the culturing course, yet the population growth patterns depend on the state of the system. Thus, these phases naturally emerge from the collective behaviour as time goes by. Figure 1 shows the population growth curve of a batch culture, and five stages can be distinguished and related to cellular activity: i) lag phase with zero growth (when the inoculum cells adapt to their new environment growing in biomass but without reproduction); ii) exponential growth phase with maximum specific growth rate (cells grow with no limitation); iii) linear phase with limited growth rate (when the progressive accumulation of ethanol and scarcity of glucose hinder cell uptake, yet do not impede cell viability and growth); iv) metabolic slowdown phase (cell viability is strongly hindered and generalized mortality takes place); v) final stage with no population growth (only a few viable cells can keep on metabolizing nutrients, mainly to preserve their biomass).

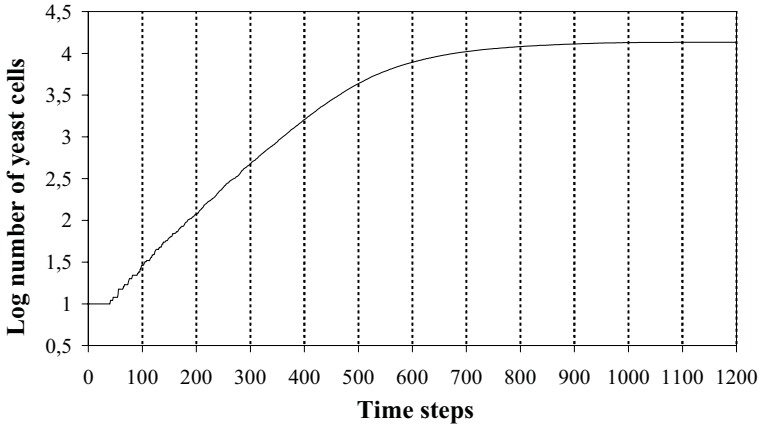

Figure 1. Time dependence of yeast cells in the simulated yeast fermentation culture.
Evolution of the yeast population: lag phase (0-40 time steps), exponential phase (40-400 time steps), linear phase (400-600 time step), metabolic slow down (600–1000 time steps), and final phase (1000-1200 time steps), approximately.

Another example of the effects of population structure on system performance, with regards to microbial activity, is the way in which the distribution of the number of bud scars among the population affects the duration of the cellular growth cycles.

The simulator saves information about every cell at each time step. This information makes possible the construction of box-and-whisker plots for different variables, in this case for the duration of the cellular cycle depending on the genealogical age of the cells (number of scars). In *S. cerevisiae* budding reproduction leads to the formation of scars in the mother cell, ridges on cell membrane left by the bud. Moreover *S. cerevisiae* cells divide asymmetrically: daughter cells, produced from the bud, are smaller than mother cells. In Figure 2 we present the durations of *Phase 1* and *Phase 2* as a function of the number of scars on the cell membrane at a fixed moment of the fermentation (time step 600). The figure shows that cells with no scars have longer *Phase 1* periods, while these periods remain more or less constant for cells with one scar or more. This can be explained through the asymmetric cell division process: before reaching their "start mass", daughter cells must increase their biomass by a greater amount. The dependence of the duration of *Phase 2* on the number of scars can also be explained in some cases using similar morphological arguments, because the surface-to-volume relation varies with cell age.

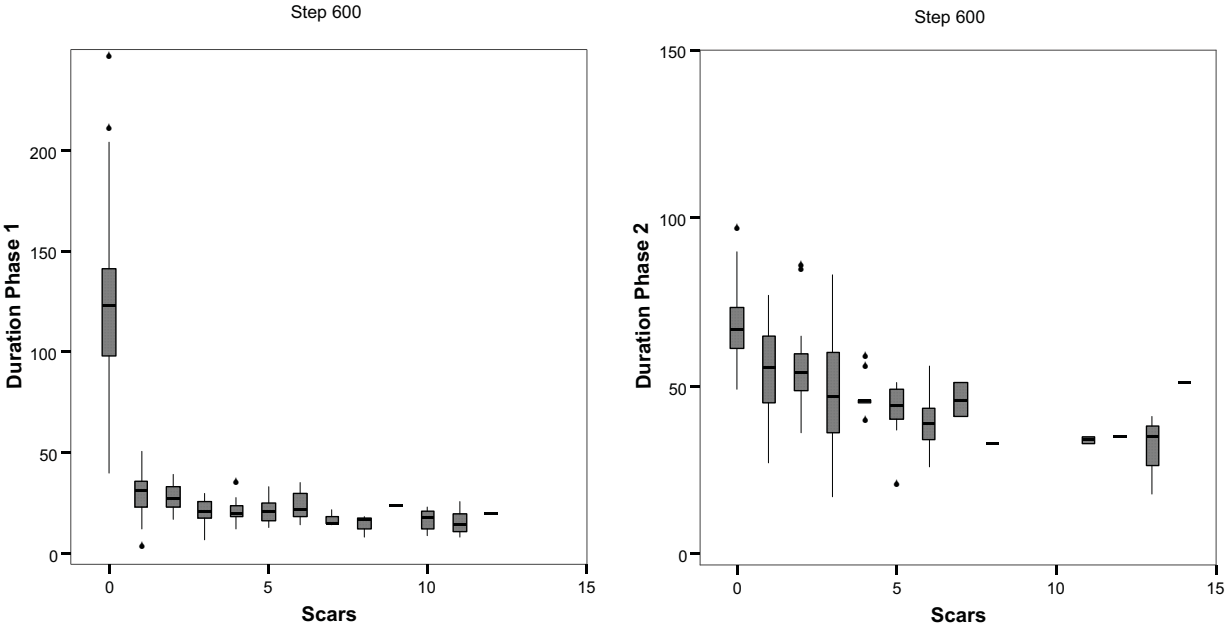

Figure 2. Boxplots of the durations of the unbudded interval (*Phase 1*) and budding interval (*Phase 2*) as a function of the genealogical ages of the yeast cells at step 600 of the simulated yeast culture shown in Figure 1.

The possibility of estimating the variation of a population, not only in number or biomass, but also in age, would favour the proposition that the production capacity in each cell depends on its cellular cycle state, its vitality and its ageing. The mathematical treatment of these cases to be integrated in kinetic models is complex and presents some difficulties. For instance, the industrial production of beer reuses yeast cropped at the end of fermentations in subsequent fermentations and it has recently been suggested that the distribution of the cell age on cropping may affect both the immediate and the long term fermentation performance. INDISIM-YEAST is an attractive tool to deal with this question due to the elements that make up the simulation model [8].

Environmental conditions and processes. If the individual yeast cells experience varying environmental conditions, then their intracellular composition or individual characteristics may consequently differ. This results in a distribution of cells with varying properties, and the distribution is determined by the environmental conditions. These individual properties can change during the course of the batch fermentation process.

Figure 3 shows the temporal evolution of the average duration of *Phase 1* for both daughter and parent cells. The use of confidence intervals allows us to indicate both the variability present in each set of data and the size of those sets, namely the data available from the simulations. The mean duration of daughter cells is consistently higher than that of parent cells; the difference increases with the evolution of the system. Both groups of cells show a smaller increase in duration of *Phase 1* during the first time steps. Thereafter it increases almost linearly; a reflection of the fact that, as time goes by, they are subjected to increasing inhibiting factors in the production of new biomass. For the parent cells the duration remains practically constant during the time steps corresponding to exponential growth. Daughter cells are more sensitive to the conditions of their environment, as they require a larger mass increase during this phase.

Despite the simplifications assumed by this model, the results achieved to date are at least in qualitative agreement with the experimental observations [19].

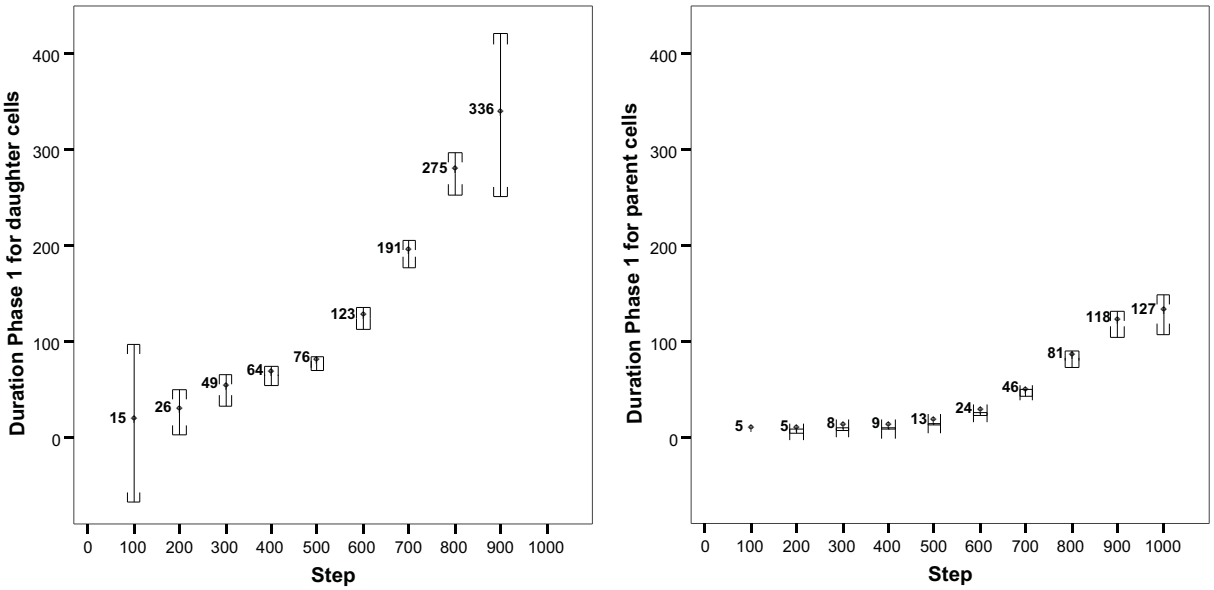

Figure 3. Temporal evolution of the 95% confidence intervals for the mean duration of the unbudded interval (*Phase 1*) of the cells in the simulated yeast batch culture shown in Figure 1, using separate plots for parent and daughter cells. The different stages of the batch culture are represented at several points: lag, where no reproduction takes place, exponential phase (100-400 time steps), linear phase (500-600 time step), metabolic slow down (700-900 or 1000 time steps), and final phase without reproduction.

External manipulations. In industrial application of fermentation processes, fed-batch operating protocols are widespread. Small variations in the operating conditions result in dramatically different behaviour of the microbial population and therefore of the bioreactor performance. This can not be easily described by unstructured or unsegregated models. This is usually solved by adding one or more correction factors to these models, leading to models of increased complexity that might include uncorrelated factors that are biologically difficult to interpret. Structured and segregated models consider the changes in the state of the cells, and may therefore provide a good description of a whole set of experiments (running under different operating conditions). These models are valuable when examining the microbial behaviour at the cellular level, but of less value to the biochemical engineer examining the performance of a fermentation process carried out in bioreactors.

The initial culture volume and glucose concentration, the feed flow rate and glucose feed concentration profiles and the final batch time are treated as decision variables in the dynamic performance optimization problem of a fed-batch culture. We have used INDISIM-YEAST to simulate fed-batch cultures operating under different conditions. In particular we modified the feeding frequency (F) and the amount of incoming glucose particles per feed (D). Figure 4 shows the ethanol production of fed-batch cultures with the same initial conditions and varying the operating regime: different combinations of frequency and amounts of glucose that result in the same quantity of glucose supplied to the system. Although the overall quantity of particles of glucose is the same in all cases, the final ethanol productions differ from case to case. INDISIM-YEAST simulations are used here as virtual experiments designed to test different proposed culturing protocols.

In conventional batch and continuous yeast cultivation systems, cell populations are randomized with respect to individual cell cycles. Non-random, or synchronous, yeast cultures are characterized by cells in the population dividing more or less in unison. The synchronization of yeast cell populations is an important experimental tool which greatly assists the biochemical analysis of cell cycle events. Synchronous yeast cultures may also have a lot of potential in biotechnology; for example, in optimizing yeast metabolite and cell cycle-modulated protein production. Following the experimental protocols or procedures for the induction of yeast synchronization, such as feeding and starving, periodic feeding or dilution rate changes in chemostat cultures [20] could be tested with INDISIM-YEAST. Many researchers have shown that continuous cultures of *S. cerevisiae* exhibit sustained population oscillations in glucose limited environments under aerobic growth conditions. Understanding and controlling this dynamic behaviour would lead to important advances in yeast production processes and could provide key insights into the cellular behaviour. A number of transient models have been proposed to explain the sustained oscillations regimes. Structured, segregated models have been developed, and a population model that considers the asymmetric yeast reproduction cycle must be incorporated to successfully account for this oscillatory behaviour [21,11]. We think that INDISIM-YEAST has in its own formulation the required elements to deal with the study of these phenomena.

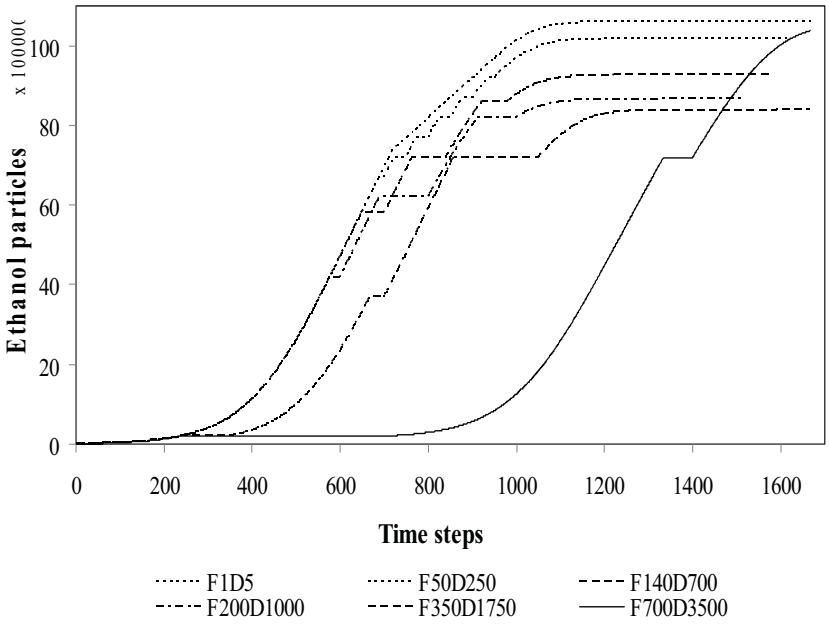

Figure 4. Temporal evolution of ethanol particles of the simulations with several Frequencies (F) of entry of substrate and densities (D) of glucose particles.

3 INDISIM-COMP

A specific simulator, INDISIM-COMP, was built in order to deal with composting process modelling and simulation [9]. It was developed taking into account the experience obtained from another INDISIM application to deal with soil organic matter, INDISIM-SOM [5]. This was the first individual simulation model that enabled us to study microbial activity in soil, to study the mineralization and immobilisation of C and N, and the integration of the nitrification process in that context assuming two different prototypes of cells, the decomposer microorganisms and nitrifying bacteria. INDISIM-COMP shares some of these ideas and incorporates new ones.

3.1 Modelling the environment

In the reported results, the composting process of 1 g organic matter during 300 hours is simulated. The space is discretized in a two-dimensional 30x30 vertical grid, with the spatial cell being the basic spatial unit. Each spatial cell has a particular composition and a local temperature that vary according to microbial activity and spatial fluxes (Figure 5). The substances considered in the system are labile organic compounds, polymerised organic compounds, resistant organic compounds, stable organic matter and other mineral compounds and gases. They are detailed in Table 1.

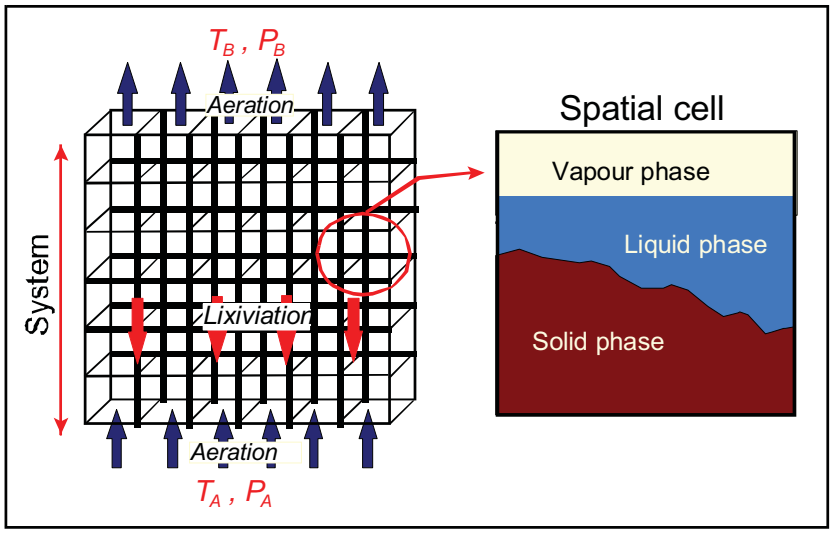

Figure 5. Spatial grid and sketch of the considered spatial processes.

Nomenclature	Group	Component	Composition
ORGANIC COMPOUNDS			
Labile carbon	C_L	Short carbon chains (e.g. monosaccharide)	$C_6H_{12}O_6$
Labile nitrogen	$(CN)_L$	Short chains with C/N=3 (e.g. amino acids)	$C_3H_7O_2N$
Polymerised carbon	C_P	Polymers in labile forms (e.g. starch, cellulose)	$n_1(C_6H_{10}O_5)$
Polymerized nitrogen	$(CN)_P$	Proteins and other compounds with C/N=3	$n_2(C_3H_5ON)$
Resistant carbon	C_R	Complex carbon chains (e.g. lignins)	$n_4(C_{20}H_{30}O_6)$
Stable organic matter	CN_{SOM}	Stable organic matter with C/N=12	$n_3(C_{12}H_xO_yN)$
MINERAL COMPOUNDS			
Ammonium	NH_4^+	-	NH_4^+
Water	$(H_2O)_{liquid}$	-	H_2O
Water vapour	$(H_2O)_{vapour}$	-	H_2O
Carbon dioxide	CO_2	-	CO_2
Oxygen	O_2	-	O_2
Ammonia	NH_3	-	NH_3

Table 1. Organic and mineral compounds considered by the model.

The environmental processes modelled in INDISIM-COMP are:

- Aeration: output flow of CO_2 and H_2O vapour, and input of O_2
- Diffusion of the soluble organic labile forms and mineral compounds in the medium
- Hydrolysis of complex compounds into labile forms, conducted by actinomycetes and fungi present in the surroundings
- Water evaporation
- Heat conduction
- Heat transfer processes linked to water evaporation and aeration

3.2 Modelling the microorganisms

Three kinds of microorganisms are considered: mesophilic bacteria, thermophilic actinomycetes and mesophilic fungi. They differ in their microbial biomass composition ($CN_{mic\text{-}bac}$, $CN_{mic\text{-}act}$ and $CN_{mic\text{-}fun}$, respectively), their shape, their optimum temperature range and their metabolism (Table 2). The microbial activity is modelled on the individual scale, taking into account the following individual actions and processes:

- *Motion:* bacteria can move from a spatial cell to a neighbouring one; actinomycetes and fungi remain fixed. The bacterial motion is given by a maximum movement radius, d_{max}, and a probability of doing so, p_{mov}.
- *Uptake:* microorganisms can uptake the substances in their spatial cells. The compounds that a certain microorganism may uptake depend on its biomass C/N relationship that must be kept. No internal reservoirs are considered. The quantity of uptaken substances is given by the medium availability, A_{ij} (j={substrate classes}, i={bac,act,fun}) and the limitations of the microbial membrane surface (thus, it depends on the microbial geometry). The resulting individual maximum uptake rate is adjusted by using the empirical Ratkowsky relationship which gives a growth dependence on temperature [17].
- *Metabolism:* it is heterotrophic and specific for each microorganism and each substrate. Table 3 summarizes the explicit metabolic pathways considered. Biological heat production is considered and specifically defined for each metabolic path. Metabolism allows the microorganisms to satisfy the individual maintenance requirements and to increase their biomass. The resultant waste products are dumped into the medium.
- *Reproduction:* the model for the reproduction cycle is based on I+C+D [1]. When the microorganisms reach a fixed mass, m_R, the division cycle begins. It has a determined duration, t_R. After this period, two individuals with similar characteristics and masses appear in the same spatial cell or in one of its surrounding cells.
- *Death and lysis:* whenever the medium conditions are adverse, the microorganisms may not be able to uptake enough nutrient to satisfy maintenance requirements. In these cases, they metabolize their own biomass to survive, and the individual mass decreases. When a specific minimum biomass is reached

the microorganism is in lysis and returns some compounds to the local medium (CN_{SOM}, NH_4^+, C_P an $(CN)_P$).

Figure 6 summarizes both the microbial and spatial models.

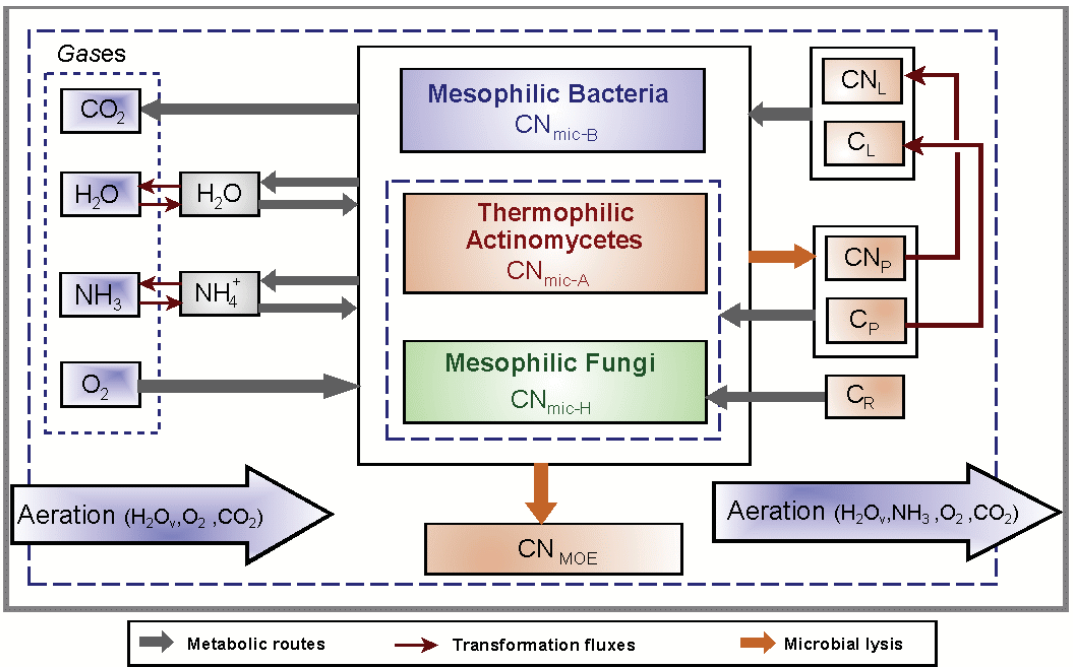

Figure 6. Sketch of the microbial model and some environmental processes.

	Mesophilic bacteria	Thermophilic actinomycetes	Mesophilic fungi
Group	CNmic-bac	CNmic-act	CNmic-fun
Composition	$C_5H_7O_2N$	$C_5H_7O_2N$	$C_{10}H_{17}O_6N$
Shape	Bacillus	Filaments	Filaments
Metabolism		See Table 3	

Table 2. Microbial differentiation in INDISIM-COMP.

Bacterial metabolism	
$CN_L + (1/3\ Y_{11})\ C_L + 6Y_{11}O_2 \rightarrow CN_{mic\text{-}B} + 6Y_{11}CO_2 + (2+6Y_{11})\ H_2O + Y_{11}Q_1$	(1)
$(5/6+Y_{12})\ C_L + NH_4^+ + (1/4+6Y_{12})\ O_2 \rightarrow CN_{mic\text{-}B} + 6Y_{12}CO_2 + (7/2+6Y_{12})\ H_2O + Y_{12}Q_1$	(2)
Actinomycete metabolism	
$CN_L + (1/3+Y_{21})\ C_L + 6Y_{21}O_2 \rightarrow CN_{mic\text{-}A} + 6Y_{21}CO_2 + (2+6Y_{21})\ H_2O + Y_{21}Q_1$	(3)
$(5/3+Y_{23})\ CN_L + (11/4Y_{23}\text{-}1/6)\ O_2 \rightarrow CN_{mic\text{-}A} + (2/3+Y_{23})\ NH_4^+ + 3Y_{13}CO_2 + (3/2Y_{23}+1)\ H_2O + Y_{23}Q_2$	(4)
$(5/6+Y_{24})\ C_P + n_1\ NH_4^+ + (1/4+6Y_{24})\ n_1O_2 \rightarrow n_1CN_{mic\text{-}A} + 6Y_{24}n_1CO_2 + (8/3+5Y_{24})\ n_1H_2O + Y_{24}n_1Q_1$	(5)
$(5/3+Y_{25})\ CN_P + (2/3\text{-}1/2Y_{25})\ n_2H_2O + (11/4Y_{25}\text{-}1/6)\ n_2O_2 \rightarrow n_2CN_{mic\text{-}A} + (2/3+Y_{25})\ n_2NH_4^+ + 3Y_{25}n_2CO_2 + Y_{25}n_2Q_2$	(6)
Fungal metabolism	
$CN_L + (7/6+Y_{31})\ C_L + (6Y_{31}\text{-}1/2)\ O_2 \rightarrow CN_{mic\text{-}F} + (2+6Y_{31})\ H_2O + 6Y_{31}CO_2 + Y_{31}Q_1$	(7)
$(5/3+Y_{32})\ C_L + NH_4^+ + (6Y_{32}\text{-}1/4)\ O_2 \rightarrow CN_{mic\text{-}F} + (7/2+6Y_{32})\ H_2O + 6Y_{32}CO_2 + Y_{32}Q_1$	(8)
$(5/3+Y_{34})\ C_P + n_1\ NH_4^+ + (6Y_{34}\text{–}1/4)\ n_1O_2 \rightarrow n_1CN_{mic\text{-}A} + 6Y_{34}n_1CO_2 + (11/6+5Y_{34})\ n_1H_2O + Y_{34}n_1Q_1$	(9)
$(10/3+Y_{35})\ CN_P + (29/6\text{-}1/2Y_{35})\ n_2H_2O + (11/4Y_{35}\text{–}13/12)\ n_2O_2 \rightarrow n_2\ CN_{mic\text{-}F} + (7/3+Y_{35})\ n_2NH_4^+ + 3Y_{35}n_2CO_2 + Y_{35}n_2Q_2$	(10)
$(1/2+Y_{36})\ C_R + n_4NH_4^+ + (2+(49/2)Y_{36})\ n_4O_2 \rightarrow n_4CN_{mic\text{-}F} + 20Y_{36}n_2CO_2 + (1+15Y_{36})\ n_4H_2O + Y_{36}n_4Q_1$	(11)

Table 3. Explicit metabolic pathways for each microorganism class and substrate.

3.3 Some simulation results

Some simulations results are presented below to illustrate the possibilities opened up by INDISIM-COMP. They should be taken as preliminary results of topics to be further investigated in depth.

Microbial activity. In composting systems there is a characteristic behaviour: the population's succession. In the first stages the mesophilic bacteria grow and their activity increases the temperature. Then, when the temperature is high enough and the labile compound concentration has decreased, the bacterial concentration decreases too and it is the turn of termophilic actynomycets and mesophilic fungi. When these populations decrease and so does the temperature, the bacteria grow again.

INDISIM-COMP simulations reproduce the population succession which is not explicitly imposed in the microbial model. The individual treatment of microbial cells allows specific modelling of each species, with specific rules. Every cell evolves individually, receiving the influence of the surrounding individuals through the neighbouring environment. The global behaviour arises from the evolution of these cells, and the population's succession is observed. In Figure 7 we can see an example of this: Figure 7a shows the evolution of the total biomass, and in Figure 7b we can distinguish among the three populations.

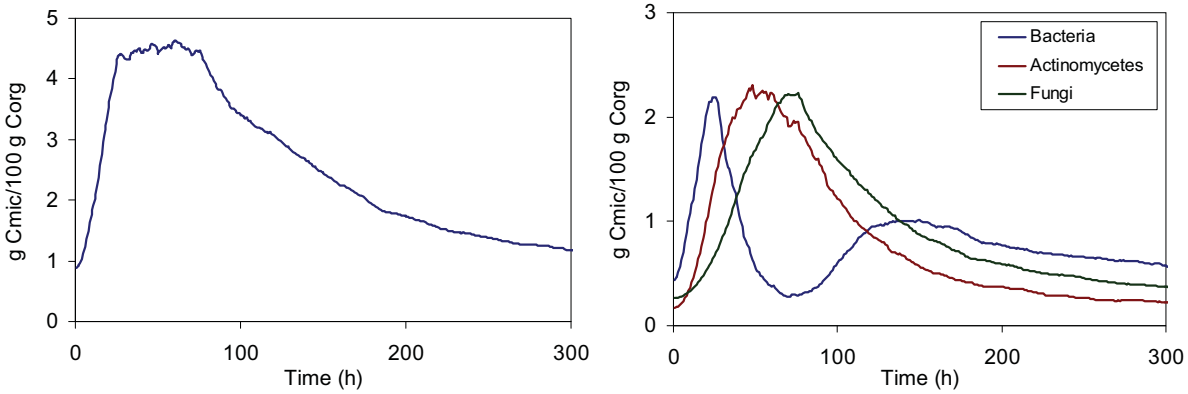

Figure 7. Simulation results that show the evolution of the global microbial population (a) and the evolutino of each kind of population (b) during a composting process.

Environmental conditions and processes. External manipulations. The simulated space is explicit: it is divided into spatial cells and we controlled the characteristics of each one. The cells interact with the medium by uptaking substrate particles and bumping into it the products of their metabolism. The simulations explicitly show the evolution of these compounds with regards to their concentration and spatial distribution. Moreover, the cells' metabolism heats the surrounding medium. This heating is modelled, as well as the heat conduction and the heat transfer through water evaporation. In Figure 8 we find an example of this: it shows the evolution of the temperature during a simulated composting process. We can observe the initial increase due to the microbial activity, followed by stabilization when the microbial activity decreases.

External manipulations may be also introduced. In Figure 8 the effect of the system aerations is shown: in this case, it was determined that an automatic aeration would take place when the temperature was over 50ºC. Thus, the observed oscillations in temperature evolution correspond to automatic aerations of the system when this condition is accomplished.

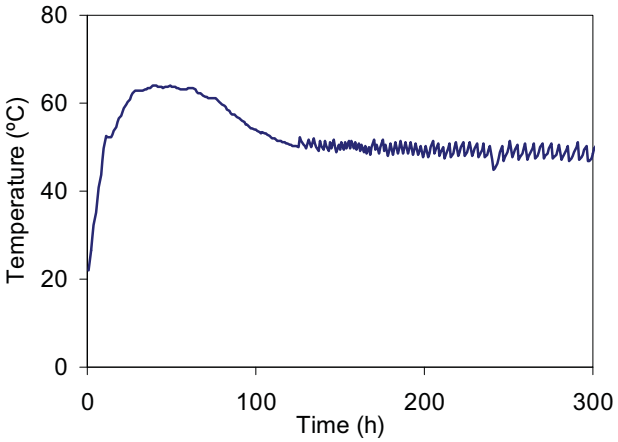

Figure 8. Evolution of the temperature in a simulated composting process.
The effect of discrete aerations is seen as discrete decreases in temperature.

4 Conclusions

We have presented two modified versions of INDISIM addressed to model (i) ethanol fermentation carried out by *S. cerevisiae* in a liquid medium and (ii) organic matter composting process carried out by complex microbial ecosystems in industrial bioreactors. Both applications are still under development, yet some conclusions can be drawn from the preliminary results presented above.

Yeast fermentation and composting processes have been investigated by a variety of mathematical models that have contributed to their control and understanding. The Individual-based approach provides a tool that covers heterogeneity in the population, stochasticity in the cellular processes and non-homogeneity or/and anisotropy in the environment. It also consists of a basic core that remains appropriate to study microbial systems operating under different external protocols. Therefore, it is a complementary approach to population-based models. The foremost contribution of INDISIM lies in its ability to deal with each microorganism at an individual level, thus providing a very direct connection between the proposed rules and their biological interpretation. It also allows the investigation of different mechanisms sepparately.

Our methodology easily predicts qualitative behaviours on a wide range of individual and population attributes which is helpful to get an idea of the relevant variables of a particular problem. INDISIM proposes mechanisms through which properties and behaviours observed in bioreactor performance emerge from properties and processes occurring at a cellular level. It also allows a detailed description of how the extracellular conditions affect the bioreactor outcome. An important advantage of the modular structure of the simulator INDISIM is that it allows exploration and modification of different features and procedures of the system under study, which permits using the same microbial model to deal with different experimental protocols. However, this methodology faces many limitations, mainly related to the difficulty in quantifying the individual microbial parameters and those that characterize the local environmental processes. It is also difficult to handle and hard to share applications, as there are no standard protocols to present, document and develop the applications.

Nevertheless, this study does not attempt to simply highlight advantages and drawbacks of the INDISIM methodology, but aims also tp provide different kinds of results that illustrate its possibilities when addressing processes of biotechnological interest.

5 Acknowledgements

We gratefully acknowledge the financial support of the Plan Nacional I+D+i of the Ministerio de Educación y Ciencia CGL2007-65142/BOS.

6 References

[1] Cooper, S.: *Bacterial growth and division.* In: Encyclopedia of Molecular Cell Biology and Molecular Medicine, Vol. 1. Wiley-VCH, 2004.

[2] Das, K., and Keener, H.M.: *Numerical model for the dynamic simulation of a large scale composting system.* Transactions of ASAE, 40 (1997), 1179 - 1189.

[3] Ferrer, J., Prats, C. and López, D.: *Mathematical Modelling Methodologies in Predictive Food Microbiology: a SWOT Analysis.* International Journal of Food Microbiology (2008), in press.

[4] Ginovart, M., López, D. and Valls, J.: *INDISIM, and individual based discrete simulation model to study bacterial cultures*. Journal of Theoretical Biology, 214 (2002), 305 - 319.

[5] Ginovart, M., Gras, A. and López, D.: *Individual-based modelling of microbial activity to study mineralization of C and N and nitrification process in soil*. NonLinear Analysis: Real World Applications, 6 (2005), 773 - 795.

[6] Ginovart, M., Xifré, J., López, D. and Silbert, M.: *INDISIM-YEAST, an individual-based model to study yeast population in batch cultures*". In: Microbiology Book Series nº 1 Vol. 1, Communicating Current Research and Educational Topics and Trends in Applied Microbiology, Formatex, Badajoz, 2007, 401 - 409.

[7] Ginovart, M. and Cañadas, J.C.: *INDISIM-YEAST: an individual-based simulator on a website for experimenting and investigating diverse dynamics of yeast populations in liquid media*. Journal of Industrial Microbiology and Biotechnology, 35(2008), 1359 - 1366.

[8] Ginovart, M., Portell, X. and Silbert, M.: *Repitching of yeast in beer fermentations: individual-based model simulations*. Submitted to MathMod 2009.

[9] Gras, A., Prats, C. and Ginovart, M.: *An individual based model to study the main groups of microbes active in composting process*". In: Modern Multidisciplinary Applied Microbiology. Exploiting Microbes and Their Interactions, Wiley-VCH, 2006, 14 - 18.

[10] Gomes, A. P., Pereira, F. A.: *Mathematical modelling of a composting process, and validation with experimental data*. Waste Management and Research, 26 (2008), 276 - 287.

[11] Henson M.A.: *Dynamic modeling and control of yeast cell populations in continuous biochemical reactors*. Computers and Chemical Engineering, 27 (2003), 1185 - 1199.

[12] Higgins, C. and Walker, L.: *Validation of a new model for aerobic organic solids decomposition: simulations with new substrate specific kinetics.* Process Biochemistry 36 (2001), 875 – 884.

[13] Kaiser, J.: *Modelling composting as a microbial ecosystem: A simulation approach.* Ecological Modelling (1996), 25 – 37.

[14] Lin, Y., and Tanaka, S.: *Ethanol fermentation form biomass resources: current state and prospects*. Applied Microbiology and Biotechnology, 69 (2006), 627 - 642.

[15] Marín, M. R.: *Alcoholic fermentation modelling: current state and perspectives*. American Journal of Enology and Viticulture, 50 (1999), 166 - 178.

[16] Mason, I. G.: *Mathematical modelling of the composting process: A review*. Waste Management, 26 (2006), 3 - 21.

[17] Ratkowsky, D. A., Olley, J., McMeekin, T. A. and Ball, A.: *Relationship between temperature and growth rate of bacterial cultures.* Journal of Bacteriology, 149 (1982), 1 - 5.

[18] Sole-Mauri, F., Illa, J., Magrí, A., Prenafeta-Boldú, F. X., and Flotats, X.: *An integrated biochemical and physical model for the composting process*. Bioresource Technology, 98 (2007), 3278 - 3293.

[19] Walker, G. M.: *Yeast Physiology and Biotechnology*. John Wiley & Sons, Chichester, 1998.

[20] Walker, G. M.: *Synchronization of yeast cell populations*. Methods in Cell Science, 21 (1999), 87 - 93.

[21] Zhang, Y., Zamamiri, A.M., Henson, M.A. and Hjortsø , M.A.: *Cell population models for bifurcation analysis and nonlinear control of continuous yeast bioreactor*. Journal of Process Control, 12 (2002), 721 - 734.