CHEMICAL ENGINEERING TRANSACTIONS

VOL. 75, 2019

A publication of AIDIC

The Italian Association of Chemical Engineering
Online at www.cetjournal.it

Guest Editors: Sauro Pierucci, Laura Piazza

ISBN 978-88-95608-72-3; **ISSN** 2283-9216

DOI: 10.3303/CET1975084

Growth Kinetics of Kefir Biomass: Influence of the Incubation Temperature in Milk

Carlos E. Barão[a,*], Suellen J. Klososki[a], Keren H. Pinheiro[a], Vanessa A. Marcolino[a], Osvaldo Valarini Junior[b], Adriano G. da Cruz[c], Thamara T. da Silva[d], Tatiana C. Pimentel[a]

[a]Instituto Federal do Paraná (IFPR) – Campus Paranavaí, Rua José Felipe Tequinha, 1400, Jardim das Nações, Paranavaí, Paraná, 87703-536, Brasil.
[b]Faculdade de Engenharia e Arquiterura (FEITEP), Avenida Paranavaí, 1164, Parque Industrial Bandeirantes, Maringá, Paraná, 87070-130, Brasil.
[c]Instituto Federal de Educação, Ciência e Tecnologia do Rio de Janeiro (IFRJ), Departamento de Alimentos, 20270-021 Rio de Janeiro, Brazil.
[d]Programa de Pós-Graduação em Ciências de Alimentos - State University of Maringa (UEM), Maringá, PR, Brazil
carlos.barao@ifpr.edu.br

Kefir is a fermented milk that can be produced from kefir grains or industrial starter cultures. It differs from other fermented milks by the performance of various types of microorganisms on the milk components. The kefir grains are gelatinous and irregular, formed by a set of microorganisms, such as yeasts and lactic acid bacteria, which are incorporated in a matrix of polysaccharides called kefiran. *Lactobacilli* make up most of the microbial population, but the total composition of the grains is not fully elucidated. There are few studies that evaluated the kinetics of kefir biomass growth at different temperatures, but the exact behavior was not explained in a consistent form, such as using mathematical models. In this context, this work aimed to improve the procedure of kefir production by studying the kinetics of the growth of kefir biomass in milk at different temperatures (298, 303 and 308 K) and characterize it by Scanning Electron Microscopy (SEM). Furthermore, the application of mathematical models to determine the growth function of the kefir biomass in relation to the time and temperature of the fermentation process was evaluated. An amount of 2% kefir grains was added to the milk and the fermentation process was carried out at 298, 303 and 308 K for 5 days. The biomass growth (increase in mass) was evaluated daily and four different mathematical models (Richards, Logistic, Gompertz and Quadratic) were applied to the data. The samples were visualized in a double-beam scanning electron microscope. The Gompertz model showed the highest values of the coefficient of correlation (R^2, 0.97-0.99) and the smallest standard deviations for the increase in the kefir grain biomass, being considered the most appropriate mathematical model. The temperature of 298 K was the most suitable for the incubation of the grains in milk, resulting in a higher increase in the biomass and a larger quantity of *lactobacilli,* coccus-like bacteria and yeast observed in the SEM. It can be concluded that 298 K may be used as the temperature for fermentation of kefir grains in milk, resulting in higher kefir biomass, which could be useful in the production of kefir fermented milks, because of the reduction in the processing time.

1. Introduction

Kefir grains are small and irregularly shaped hard granules with yellow or white color and resembling cauliflower blossoms (Figure 1). Kefir grains are composed of a complex mixture of bacteria and yeasts that coexist in a symbiotic environment. They are used to produce kefir fermented milks, which are slightly carbonated beverages with a typical yeast flavor (Apart et al., 2017).
The traditional method for producing kefir fermented milk is to inoculate 1 to 10% of the grains in milk at room temperature (298 K) for a fermentation period of 18 to 30 h, being 24 h of fermentation the most common fermentation time (Farnwoth and Mainville, 2008; Rosa et al., 2017). However, the increase in the biomass is

Paper Received: 22 June 2018; Revised: 25 October 2018; Accepted: 4 April 2019

Please cite this article as: Barao C.E., Klososki S.J., Pinheiro K.H., Marcolino V., Valarini Junior O., Cruz A.G., Da Silva T.T., Pimentel T., 2019, Growth Kinetics of Kefir Biomass: Influence of the Incubation Temperature in Milk, Chemical Engineering Transactions, 75, 499-504 DOI:10.3303/CET1975084

sometimes low, therefore, to use the kefir grains in commercial applications the biomass production must be improved (Apar et al., 2017). The increase in biomass allows continuous production and reduce the processing time (Rosa et al., 2017, Barukcic et al., 2017). Furthermore, it allows the utilization of kefir grains in other applications, such as polysaccharide production (Gorsek and Tramsek, 2008). The fermentation conditions are considered the main factors that impact on the growth of the different type of microorganisms (yeast, lactic acid bacteria and acetic acid bacteria) present in kefir grains (Arslan, 2014). Previous studies have evaluated the impact of the fermentation parameters on the quality of the kefir fermented milks (Januário et al., 2016, Barukcic et al., 2017). In this study, the attention was placed on maximum biomass increase, and not on the quality of the kefir fermented milk.

Figure 1: Kefir grains

In the field of predictive microbiology, mathematical models have been used to describe the behavior of the microorganisms under different conditions, such as temperature, fermentation time, pH and water activity (Zwietering et al., 1990). The growth changes usually result in a sigmoid-shaped curve and several growth models can be used to describe this type of curve (Lopez et al., 2004), most of them are based on mathematical equations such as Logistic, Gompertz and Richards (Gorsek and Pecar, 2016). The incorporation of mathematical models into the process development can reduce the costs related to the design of industrial plants for kefir grain mass production (Tramsek and Gorsek, 2008).

There are few studies that evaluated the kinetics of kefir biomass growth at different temperatures, but the exact behavior was not explained in a consistent form, such as using mathematical models. In this context, this work aimed to improve the procedure of kefir production by studying the kinetics of the growth of kefir biomass in milk at different temperatures (298, 303 and 308 K) and characterize it by Scanning Electron Microscopy (SEM). Furthermore, the application of mathematical models to determine the growth function of the kefir biomass in relation to the time and temperature of the fermentation process was evaluated.

2. Material and methods

2.1 Kefir grain activation

Kefir grains were obtained by a private household in the city of Curitiba, Paraná, Brazil. For activation, the grains were added to full-fat milk (Nestlé®, 3% fat) and incubated at 298 K for 24 h. The grains were separated from the fermented milk with a sieve, reinoculated into milk and incubated at 298 K for 24 h. This procedure was repeated three times for subsequent three days. Then, the grains were considered active and were used as starter inoculum.

2.2 Study of the growth kinetics of kefir biomass

Activated kefir grains (2%) were added to the milk (Nestlé®, 3% fat) and the fermentation process was carried out at 298, 303 and 308 K for 5 days in a Biochemical Oxygen Demand (BOD). The concentration of the grains was defined in previous study (Januário et al., 2016), in which 2% grains and 24 h of fermentation resulted in kefir fermented milks with the most suitable physicochemical and sensorial characteristics.

Kefir grain mass was determined, in duplicates, by weighing on an analytical balance. For that, the kefir grains were separated from the fermented milk with a sieve, washed with milk, dried on a paper towel and weighed using an analytical balance (Apar et al., 2017). After determining the grain mass, the grains were returned to the milk. This procedure was repeated daily for 5 days.

2.3 Modelling Studies

Kinetic parameters were estimated and adjusted using a non-linear regression technique in order to fit with the experimental data. Mathematical models of the kefir biomass growth function were applied in relation to the time and temperature of the fermentation process. Four mathematical models were analyzed (Table 1) (Apar et al., 2017).

Table 1: Mathematical models

Model	Equation
Richards	$y = \frac{a}{(1 + e^{b-cx})^{1/d}}$
Logistic	$y = \frac{a}{1 + be^{-cx}}$
Gompertz	$y = ae^{-e^{b-cx}}$
Quadratic	$y = a + bx + cx^2$

2.4 Scanning electron microscopy

The kefir grains were cut in small blocks (size length: approx. 1-2 mm) and fixed overnight with 2% glutaraldehyde in 0.1 M sodium phosphate buffer (pH7.2). After that, they were dehydrated (in graded series of ethanol), critical point dried with liquid carbon dioxide, deposited over one side of a commercial double-coated adhesive tape, and attached to a stub. Then, they were coated with gold in a sputter coater (SCD 050, Bal-Tec, Wetzlar, Germany). Before critical point drying, the samples were transferred to microporous specimen capsules. The specimens were viewed with a scanning electron microscope (Quanta 250, Thermo Fisher Scientific-FEI, Waltham, MA, USA) at an acceleration voltage of 15 kV.

3. Results and Discussion

3.1 Study of the growth kinetics of kefir biomass

Figure 2 presents the mass increase of the Kefir grains during the fermentation period (5 days).

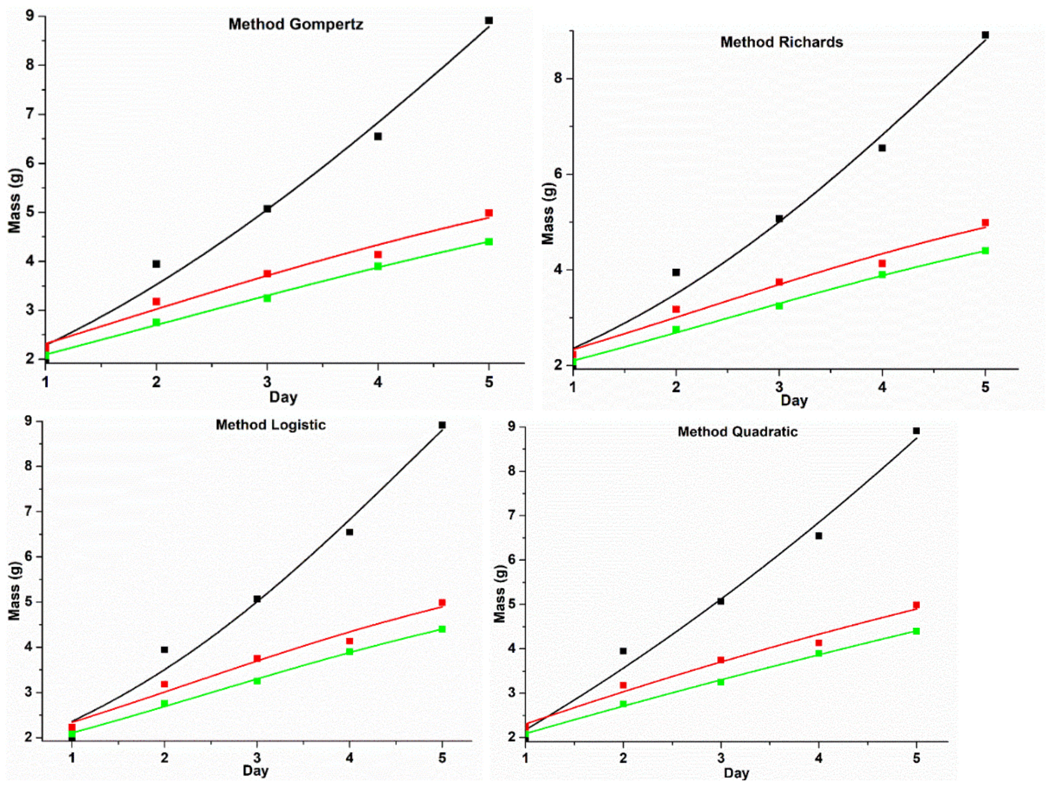


Figure 2: Mass increase of the kefir during the fermentation period (5 days). (■) T = 298 K; (■) T = 303 K; (■) T = 308 K.

The temperature of 298 K was the most suitable, as it resulted in the highest increase in the production of kefir biomass on all the studied days. At the end of the 5-day period, an increase of about 445% (~ 2g - 8.9 g) was observed at the temperature of 298 K, while temperatures of 303 and 308 K showed an increase of about 223% (~ 2g - 4.9 g) and 221% (~2g - 4.4 g), respectively. The temperature is one of the factors that play a major role in kefir production and, together with the fermentation time, impacts directly on the characteristics of the kefir fermented milk (Sarlak et al., 2017). The results indicate that the temperature of 298 K contributed to the proliferation of the microorganisms in the milk during fermentation (Gorsek and Zajsek, 2010). The reduced kefir growth at temperatures above 298 K can be related to kefir grain denaturation (Gorsek and Pecar, 2016), water loss of the grains (Apar et al., 2017) or reduced proliferation of the microorganisms.
Table 2 presents the estimated parameters and the correlation coefficients for the different models at the studied temperatures.

Table 2: Estimated parameters and Correlation coefficients (R^2) for the different model at the studied temperatures

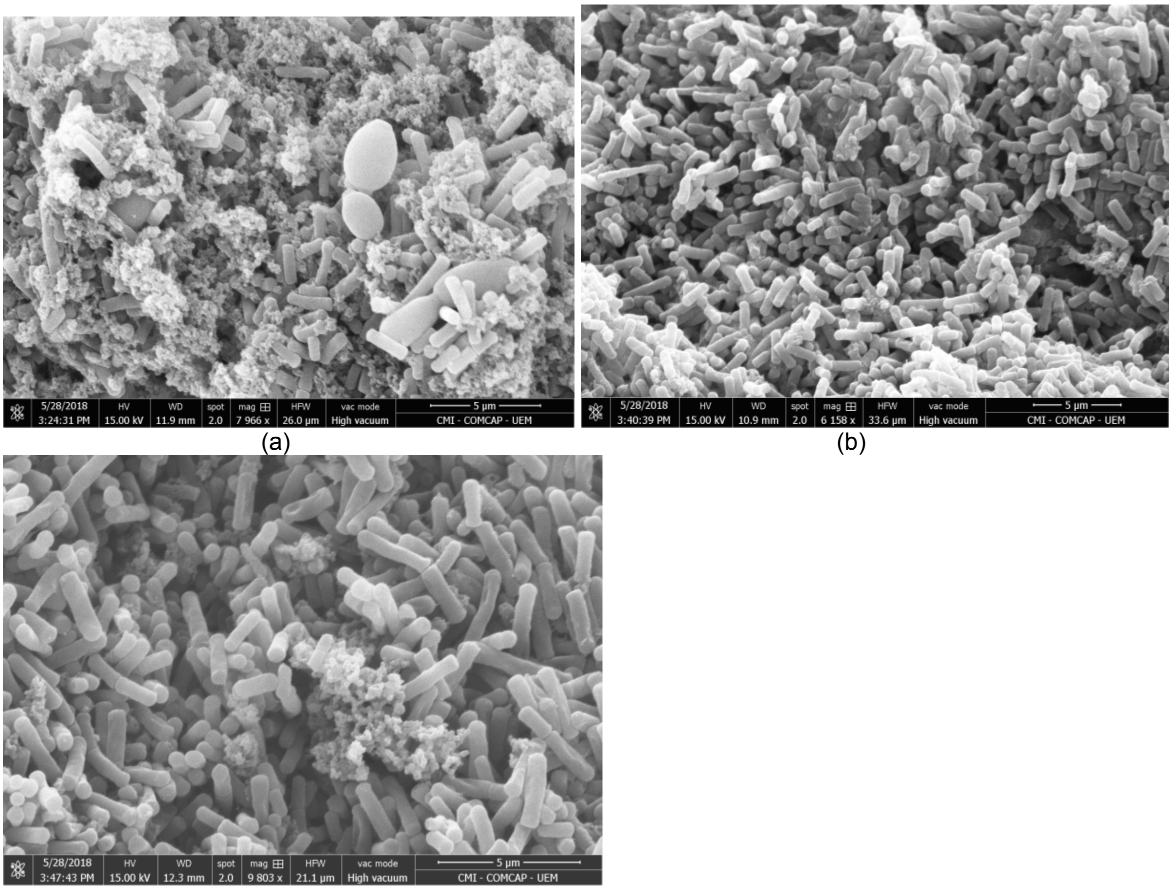
Methods	T (K)	a ± σ	b ± σ	c ± σ	d ± σ	R^2	*p*-valor
Richards[1]	298	16.74 ± 40.11	58.65 ± 2.2 x10^4	12.27 ± 5.0x10^4	25.69 ± 9004.20	0.94	0.074
	303	6.40 ± 47.90	0.68 ± 911.21	0.30 ± 1.4 x10^5	0.69 ± 924.50	0.91	0.053
	308	5.97 ± 10.63	1.19 ± 382.68	0.48 ± 152.44	1.17 ± 375.25	0.99	0.017
Logistic[2]	298	16.74± 11.62	9.80 ± 5.30	0.48 ± 0.19	-	0.96	0.003
	303	6.40 ± 1.95	2.67 ± 0.72	0.43 ± 0.19	-	0.96	0.002
	308	5.97 ± 0.66	2.76 ± 0.28	0.41 ± 0.06	-	0.99	0.001
Gompertz[3]	298	31.19± 44.70	1.14 ± 0.36	0.18 ± 0.15	-	0.97	0.003
	303	7.27 ± 3.18	0.40 ± 0.19	0.26 ± 0.16	-	0.96	0.002
	308	7.27 ± 1.34	0.44 ± 0.09	0.23 ± 0.05	-	0.99	0.001
Quadratic[4]	298	0.96 ± 0.83	1.13 ± 0.63	0.08 ± 0.10	-	0.97	0.003
	303	1.53 ± 0.41	0.80 ± 0.31	-0.02 ± 0.05	-	0.96	0.001
	308	1.45 ± 0.11	0.65 ± 0.09	-0.01 ± 0.01	-	0.99	0.001

[1]a=g; b=none; c=1/day; d=none; [2]a=g; b=none; c=1/day; [3]a=g; b=none; c=1/day; [4]a=g; b=g/day; c=g/day^2.

The highest values of the correlation coefficient (R^2) were found for the Gompertz and Quadratic models (0.97 at 298 K, 0.96 at 303 K and 0.99 at 308 K). The Gompertz model was considered the most adequate model to represent the biomass increase as a function of fermentation time and temperature, since the highest value for the correlation coefficient was a *p*-value that is significant for the sample (*p*-value < 0.05) (Table 2). Richards model presents the highest values for the standard deviation (Table 2). In addition, this equation has no significance (*p*-value > 0.05) to represent the growth data of kefir in milk. Probably this is because the data have a linearity up to day 5. A model with lower standard deviation is considered more statistically adequate to describe a growth curve (Tramsek and Gorsek, 2008). In addition, a three-parameter model has advantages over a four-parameter model (if sufficient to describe the data), such as: (1) it is easier to use, (2) its solution is more stable, as the parameters are less correlated and (3) estimates have more degrees of freedom, which is important when there are limited measurement points (Zwietering et al., 1990).

3.2 Scanning electron microscopy

The distribution and diversity of the microbial population of kefir grains were observed in a scanning electron microscope. The micrographs showed that the prevalent microbiota was of bacilli (short and curved long) cells (Figure 3), as reported by Akal et al. (2018). In fact, the kefir grains are composed of lactic acid bacteria (10^8 CFU/g), acid acetic bacteria (10^5 CFU/g) and yeast (10^6-10^7 CFU/g) (Prado, 2015). The presence of *Lactococcus* could not be observed in any formulation, which could be related to the bad attachment of this microorganism to the grain (Gao and Li, 2016).
The temperature of 298 K (Figure 3a) resulted in products with a higher diversity of microorganisms, corroborating the results observed for mass increase (Figure 2). In this formulation, it was observed different types of microorganisms, some of them resembling yeasts and other presenting formats of bacillus. As the temperature increased, only *Lactobacillus* could be observed (Figures 3b and 3c). Probably, at temperatures above 298 K there is a decrease in the counts of some microorganisms, mainly yeasts. In fact, Gorsek and Zajsek (2010) reported increases in the count of yeasts in temperatures between 292 and 298 K and a progressive decrease in temperatures higher than 298 K, suggesting quick deaths at higher temperatures.



(a) (b)

(c)

Figure 3: Scanning electron microscopy of kefir grains at three temperatures (a) 298 K, (b) 303 K and (c) 308 K.

The lower number of *Lactobacillus* in the products fermented at 298 K can be explained by inhibition properties. Yeasts, when incubated at suitable temperatures, produce high levels of gas and alcohol, which inhibit the growth of lactic acid bacteria (Grosek and Zajsek, 2010). Furthermore, *Lactobacillus* has optimum temperature at 35-37 ^{o}C, which can explain its prevalence at highest temperatures (303 and 308 K).

Kefiran is the main polysaccharide of the kefir grains and has been used as a food gum in the food industry, in the development of food packages and in fortified products, due to its health effects (Guzel- Seydim et al., 2011). It was observed that the grains fermented at a temperature of 298 K (Figure 3a) presented a higher concentration of kefiran, suggesting that the increase in the kefir grain biomass positively affect the kefiran production.

4. Conclusion

Temperatures of 298 K should be used for fermentation of milk with kefir grains, resulting in a higher production of biomass and more diversity of microorganisms. Furthermore, fermentations at this temperature may improve the production of kefiran, an important ingredient for the food industry. Gompertz equation could be used to represent the increase in kefir grain biomass as a function of time and temperature of fermentation for the conditions analyzed in the present study. It can be concluded that mathematical models can be applied as a tool in the development of processes and the most suitable temperature for fermentation of kefir grains in milk is 298 K.

Acknowledgment

This research was supported by Federal Institute of Paraná (PROAP and PIAP), CNPq (Brazilian National Council for Scientific and Technological Development and FAPPR (Araucaria Foundation).

References

Akal C., Budak S. Ö, Yetisemiyen, A, 2018, Potential Probiotic Microorganisms in Kefir, Chapter In: Budak, S. Ö, Akal, C., Microbial Cultures and Enzymes in Dairy Technology, IGI Global, Hershey, USA, 276-296

Apar D. K. et al., 2017, Kefir grain biomass production: influence of different culturing conditions and examination of growth kinetic models, Journal of Food Process Engineering, 40, e12332.

Arslan S., 2014, A review: chemical, microbiological and nutritional characteristics of kefir, CyTA - Journal of Food, 13(3), 340-345.

Barukčić, I., Gracin L., Jambrak A. R., & Božanić R., 2017, Comparison of chemical, rheological and sensory properties of kefir produced by kefir grains and commercial kefir starter. Mljekarstvo 67(3), 169–176.

Farnworth E. D. & Mainville A., 2008, Kefir-A fermented milk product. 2a ed. Taylor & Francis, p. 89-128.

Gao X., & Bo L., 2016, Chemical and microbiological characteristics of kefir grains and their fermented dairy products: A review. Cogent Food Agric, 2:127–152.

Gorsek A., Pecar D., 2016, Propagation of natural starter culture in whey: optimization and kinetic study, Chemical Engineering Transactions, 49, 481-486.

Gorsek A., & Tramsek M., 2008, Kefir grains production: An approach for volume optimization of two-stage bioreactor system, Biochemical Engineering Journal, 42, 153–158.

Gorsek A., & Zajsek K., 2010, Influence of temperature variations on ethanol production by kefir grains - mathematical model development, Chemical Engineering Transactions, 20, 181-186.

Güzel-Seydim Z., Kok-Tas T., Ertekin-Filiz B., Seydim A.C., 2011, Effect of different growth conditions on biomass increase in kefir grains, J. Dairy Sci. 94 (3), 1239-1242.

Januário J. G. B., De Lima T. M., Portella D. A. C., Januario C. B., Klososki S. J., & Pimentel T. C., 2016, Development of Kefir beverages: Standardization of process parameters, Brazilian Journal of Food Research, 7(2), 80-95.

Lopez S., Prieto M., Dijkstra J., Dhanoa M.S., France J., 2004, Statistical evaluation of mathematical models for microbial growth, International Journal of Food Microbiology, 96: 289–300

Mistry M. V., 2004, Fermented Liquid Milk Products. In: HUI, Y. H.; et al. Handbook of Food and Bevarage Fermentation Technology. New York: Marcel Deker, Cap. 45.

Prado M. R., Blandon L. M., Vandenberghe P.S., Rodrigues C., Castro G. R., Thomaz-Soccol V., & Soccol C. R., 2015, Milk kefir: Composition, microbialcultures, biological activities, and related products, Frontiers in Microbiology, 6,1–10.

Rosa D. D., Dias M. M. S., Grześkowiak L. M., Reis S., Conceição L. L., Peluzio M. C., 2017, Milk kefir: nutritional, microbiological and health benefits, Nutrition research reviews, 30(1), 82-96.

Sarlak T., Moslehisahd M., Akbari-Adergani B., Salami M., 2017, Effects of Starter Culture and Storage Temperature on Functional, Microbial and Sensory Characteristics of Kefir during Storage, Journal of Pharmaceutical & Health Sciences, 5(1), 23-35.

Tramsek M. & Gorsek A., 2008, Analysis of growth models for batch kefir grain biomass production in RC1 reaction system, J. Food Process. Eng., 31, 754–767

Zwietering M. H., Jongenburger I., Rombouts F.M., Van't Riet K., 1990, Modelling of the Bacterial growth curve, Applied and Environmental Microbiology, 56, 1875-1881.