Poster Session III

Bioprocess and Bioengineering with Acetic Acid Bacteria

PIII-1: Kinetics for Continuous Surface Fermentation of Acetic Acid Bacteria

A.S. Mori[1*], Y. Suneya[2], N. Nakamura[2]

[1]*Dept. Chemical Engineering., Faculty Engineering, Niigata Univ., Nishi-ku Niigata-shi, Niigata 950-2102, Japan (*Present address : Mori Akihiko Consulting Engineer's Office, Hachioji-shi, Tokyo 192-0352, Japan), [2]Kewpie Jyozo Co.,Ltd., Fuchu-shi, Tokyo 183-0034, Japan*
E-mail:stephano.mori@nifty.ne.jp

Yasui, *et al.* succeeded continuous surface vinegar making process connected gutter type fermenters.. But kinetics of such fermentation was not reported about this. Authors would like to make report as we worked a little experiments devise of continuous surface fermentation for vinegar,

Microorganism used and medium used was same as the batch fermentation. Fermenter used was made from poly vinyl chloride and double box type, outer vessel used as water bath, and inner vessel, 200 mm long, 720 mm wide, and 220 mm high, with brim which was put on an outer vessel, and used as fermenter. Upper side of inner vessels was closed tightly with glass plates, air was naturally aerated through aeration hole with diameter of 19 mm on the side of upper part of vessels. Temperature was kept at 34°C by immersing in water bath with thermostat. Fermenting liquid flowed in the side of vessel and flowed out from overflowing pipe set on another side of the vessel. The vessels was put up the frame of iron by multistory and overflowing pipe connected with inflow pipe of the next vessel. Medium flowed in first vessel from feed tank by metering pump, under the first vessel fermenting liquid flow down naturally. Every vessel had 4 dam plates, top of which had an aperture of 1 or 2 mm in the vessel. Inoculation to fermenting surface of all vessels was done with pellicles of Acetic Acid Bacteria and fermentation started. After arrived assumed acidity, medium and fermenting liquid started flowing. During fermentation once every day, treatment was conducted to remove fat pellicles. At the finishing all pellicles were gathered with net and was measured dry weight as total biomass $|_m$. Removed pellicles was measured by dry weight as surplus biomass χ_S (g/dm^2). Acetic acid and alcohol concentration determined same as batch fermentation.

In the continuous surface fermentation mass balance of ethyl alcohol as substrate and acetic acid as products was given by following equations resulting fermentation in complete mixing tank.

$$u_{pt} = r_{pt} / S_v = D_t (P_f - P_0) / S_v \cdots (1) \qquad u_{SAt} = r_{SAt} / S_v = Dt (S_{A0} - S_{Af}) / Sv \cdots (2)$$

But, u_{pt} is a total production rate per unit area, r_{pt} is a total production rate per unit volume, S_v is specific area (fermentation area/volume, dm^{-1}), D_t is total dilution rate, P_f, P_0 is acetic acid concentration (g/L) of entrance or exit, u_{SAt} is total substrate (alcohol) consumption rate per unit area,

r_{SAt} is total substrate consumption rate per unit volume, S_{A0}, S_{Af} is alcohol concentration (mL/L) of entrance or exit. Surplus biomass removing rate $\Delta\chi_S/\Delta t$ was made to act growth rate, and resulted not large error on the average value for long-term. The growth rate per unit area was constant regardless of depth of liquid, and was correlated positively to acid production rate per unit area. Acid production rate per unit biomass was positively correlated to depth of liquid, but at 8 cm of depth had maximum value, and declined over that depth. Acetic acid concentration and ethyl alcohol concentration of exit was correlated to medium feed rate per unit area positively or negatively respectively, but over about 7 L/h・m both concentrations became constant level. Accumulated acetic acid was correlated to integral cell mass positively, and correlated hyperbolically to total dilution rate.

The growth activity μ and depth of liquid were correlated with like a monomolecular reaction and μ and production activity ρ was correlated positive linearly. Although continuous surface fermentation is producible only vinegars with low acidity, acid production rate showed several hundred times high than Generator process.

PIII-2: Kinetics for Batch Surface Fermentation of Acetic Acid Bacteria

A.S. Mori[1*], Y. Suneya[2], N. Nakamura[2]

[1]*Dept. Chemical Engineering, Faculty Engineering, Niigata Univ., Nishi-ku Niigata-shi, Niigata 950-2102, Japan (*Present address : Mori Akihiko Consulting Engineer's Office, Hachioji-shi, Tokyo 192-0352, Japan),* [2]*Kewpie Jyozo Co.,Ltd., Fuchu-shi, Tokyo 183-0034, Japan*
E-mail:stephano.mori@nifty.ne.jp

Surface fermentation of vinegar is one of the oldest professional method for making seasonings, known as ceramic pots mashing like traditional techniques about the Kyusyu district in ancient times. At present time, many vinegar makers use box type fermenter for commercial vinegar making. Yasui, *et al.* succeeded continuous vinegar making process connected gutter type fermenters.. But kinetics of surface fermentation which is a theoretical basics of many techniques has not knowledge about this. Authors would like to make report as we worked model experiments of batch surface fermentation for vinegar,

Microorganism used was *Acetobacter acti* K1001 strain isolated from industrial mash of factory of Kewpie Jyozo Co., Ltd.. Medium used as standard medium consisted of alcoholic fermented liquid of wort 400 mL, denatured alcohol (alcohol content 49.5%) 34 mL, distilled vinegar (acetic acid concentration 115 g/mL) 60mL, and water in 1L. Fermenter used was made from poly vinyl chloride and box type, 250 mm long, 396 mm wide, and 205 mm high, divided 2 chamber by a partition plate, both chambers had made possible two kinds of experiments. Upper side of chambers was closed tightly with glass plates, air was naturally aerated through aeration hole with diameter of 19 mm on the side of upper part of chamber. Temperature was kept by immersing in water bath with thermostat the whole chamber. Inoculation into fermenting chamber use a medical spoon to scoop pellicles of Acetic Acid Bacteria which was precultured with standard medium and it was floated over the medium of the chamber and it spread and stretched over the whole surface of a chamber of main culture and then fermentation started. Only sampling fermenting liquid was conducted to avoid demolition of pellicles during fermentation, at the finishing all pellicles was gathered with net and was measured dry weight as total biomass. Acetic acid concentration P (g/L) was titrated by 1/4 mol NaOH with phenolphthalein as an indicator, and converted acetic acid. Alcohol concentration S_A(mL/L) was sampled 25mL of examined liquid, neutralize by 30% NaOH, distillated more than 90 % of total volume of distillate, recovered to 25 mL, and its refractive index was read by immersion refract meter (Carl Zeis) and convert to ethyl alcohol concentration with the conversion table.

Dissolved oxygen concentration in the fermenting liquid of this culture was extremely low, such as 1.6 % of air saturated medium at depth of liquid, and then bacteria existed except for surface of liquid were ignored. The optimum temperature for cultivation was 34 °C. The maximum amount of biomass per unit areaχ_m, and maximum accumulated amount of acid $(\Delta\Phi)_m$ was linearly correlated, respectively. The growth activity μ and production activity ρ was connected, μ and ρ was correlated positive linearly. Production yield Y_P was almost constant. The maximum amount of product activity was recognized at depth of fermented liquid z of about 4.3 cm, but when volume efficiency rp was considered best advantageous depth of fermenting liquid was 3.5 cm. However, when another condition constant, the production capacity was proportional to fermenting surface area A, in case of constant surface Aconst, the larger depth of liquid was, the higher the capacity was found.

PIII-3: MODELLING, SCALE UP AND DESIGN OF PROCESSES IN AERATED-STIRRED FERMENTORS FOR INDUSTRIAL PRODUCTION OF VINEGAR

José-María González-Sáiz[a], Diego Garrido-Vidal[a], Nuria Pérez-del-Notario[b], Consuelo Pizarro[b]

[a] *Chemical Engineering. Department of Chemistry. University of La Rioja. C/ Madre de Dios 51. 26006 Logroño, La Rioja (Spain).*

[b] *Analytical Chemistry. Department of Chemistry. University of La Rioja. C/ Madre de Dios 51. 26006 Logroño, La Rioja (Spain).*

E-mail: consuelo.pizarro@unirioja.es

The production of vinegar in an aerated-stirred reactor has been modelled using a hybrid approach. The growth rate is modelled according to the total biomass specific growth rate, $\mu_g \nu$, which represents the growth of an active biophase and the fraction of cells which are growing. A quadratic model based on the process variables, i.e. hydrostatic pressure, ethanol concentration, aeration, agitation and temperature has been developed for $\mu_g \nu$. The substrate and product kinetics are related mechanistically to the growth rate, including the kinetics of acetoin and ethyl acetate production. The model has been completed with the models for oxygen transfer and liquid gas transfer. The prediction capacity is validated by means of simulations of semi-continuous and continuous real processes developed in a pilot fermentor.

Once the model has been developed, a scale-up methodology has been designed and applied. The response surfaces generated by the model demonstrates that aerated mechanical power input, superficial air velocity, temperature, hydrostatic pressure and concentrations of compounds must be kept in the industrial scale fermentors with the same geometry dimensions of the pilot fermentor. These variables maintain the values $k_L a$, $C^*_{O_2,L}$ and $OTR_{\max}$ and asses the same effects of the hydrodynamics and oxygen transfer, allowing to scale down the values of the process variables of the industrial scale fermentors to the values of the pilot fermentor, which are required for applying the models. On these basis, a Simulation Envinronment for Acetification Processes (SEAP) has been developed to design industrial processes with any scale and predict the behaviour of the fermentations and the costs of the production of vinegar. Finally, an example of design and optimization of a fermentation process with SEAP is reported, applying an optimization methodology based on response surfaces and genetic algorithms using a desirability function as response.

PIII-4: Structural Identifiabilitiy of a Model For The Wine Acetic Acid Fermentation Process

J.E. Jiménez-Hornero[1], I.M. Santos-Dueñas[2], J.L. Bonilla-Venceslada[2], C. Álvarez-Cáliz[2], J.C. Mauricio[3], I. García-García[2],

[1]*Computing and Numerical Analysis,* [2]*Chemical Engineering,* [3]*Microbiology Departments, University of Cordoba, PO Box 14071 Cordoba, Spain.*
E-mail: jjimenez@uco.es

Modelling of a process is a key stage previously to its optimization and control Parameter estimation represents one of the most important and critical stages in model development. In this way, it is important to know, in advance, whether these parameters can be uniquely determined, in such a way that estimation results allow physical conclusions to be inferred. The model structure as well as the quantity and quality of experimental data must be considered in this analysis. This study is known as identifiability and presents two different aspects: structural or theoretical identifiability and practical one. The former only takes into account the complexity of the model structure for a given input-output behaviour[1], whereas the latter, besides the model structure, considers the quality and quantity of experimental data. This work is focused on the first of these concepts.

Whereas for linear models, theoretical identifiability is a well known issue that can be analysed using several reliable tests[2], for non-linear models the problem is much more complex and there are relatively few methods for. Normally, these methods require software tools for symbolic computation. Basically, they can be classified into analytical methods[3,4,5,6,7] and numerical ones[8,9,10]. Because of the difficulty in using analytical methods for practical problems, numerical methods are normally used for assessing the theoretical local identifiability and, eventually, the global one.

When analytical methods were applied to a new model[11] for the wine acetic acid fermentatiton, no conclusion could be obtained about its structural identifiability; nevertheless, application of numerical methods was easier than previous ones but only could be used for analysing local structural identifiability. Only a method based on the similarities between the structural identifiability and the observability concept[10], provided positive results concluding that the model was structurally locally identifiable.

Acknowledgements
The authors are grateful to Spain's Ministry of Education and Science for funding this research (AGL2002-01712, PET2006_0827), and also to Grupo SOS, Spain for their invaluable help and information.

1. Bellman, R. and Aström, K.J., ***Math. Biosci***., **7**, 329-339 (1970).
2. Cobelli, C. et al., ***Math. Biosci***., **44**, 1-18 (1979).
3. Pohjanpalo, H., ***Math. Biosci***., **41**, 21-33 (1978).
4. Walter, E. and Lecourtier, Y., ***Math. Comput. Simulation***, **24**, 472-482 (1982).
5. Vajda, S. et al., ***Math. Biosci***., **93**, 217-248 (1989).
6. Tunali, E.T. and Tarn, T.J., ***IEEE Trans. Automat. Control***, **32**. 146-154 (1987).
7. Ljung, L. and Glad, T. ***Automatica***, **30**, 265-276 (1994).
8. Braems, I. et al., ***Proceedings of the 40th Conference on Decision and Control*** (2001).
9. Gerdin, M., ***Proceedings of the 14th IFAC Symposium of System Identification*** (2006).
10. Sedoglavic, A., ***J. Symbolic Comput***., **33**, 735-755 (2002).
11. Jimenez-Hornero, J. E. et al., ***The 2nd Int. Conf. on Acetic Acid Bacteria*** (2008)

PIII-5: A New Proposed Model For The Acetic Acid Fermentation Process

J.E. Jiménez-Hornero[1], I.M. Santos-Dueñas[2], J.L. Bonilla-Venceslada[2], S. Baena-Ruano[2], J.C. Mauricio[3], I. García-García[2].

[1]*Computing and Numerical Analysis,* [2]*Chemical Engineering,* [3]*Microbiology Departments, University of Cordoba, PO Box 14071 Cordoba, Spain*
E-mail: jjimenez@uco.es

The social and economic importance of vinegar justifies the interest and research currently carried out about its different aspects. Modelling is a key stage when optimization of the process is one of the main aims. Different proposals of kinetic equations can be found at bibliography, trying to show the influences detected at experimentation[1-9]. Nevertheless, a series of experiments carried out under similar operational mode to that used at industry (but at a bench scale), may justify the proposal of a new kinetic equations set that takes into account the ethanol and acetic acid influences over the process, as well as a new equation for cell lysis, as far as we know not introduced previously.

In addition to the usual mass balances for structured models, the model introduced in this work presents the following new kinetic equations:

$$r_{X_c} = \mu_{\max} \cdot f_e \cdot f_a \cdot f_o \cdot X_v \qquad f_e = \frac{E}{E + K_{SE} + \frac{E^2}{K_{IE}}} \qquad f_a = \frac{1}{1 + \left(\frac{A}{K_{IA}}\right)^4} \tag{1}$$

$$r_{X_d} = \mu_d^0 \cdot f_{dE} \cdot f_{dA} \cdot X_v \qquad f_{dE} = 1 + \left(\frac{E}{K_{mE}}\right)^4 \qquad f_{dA} = 1 + \left(\frac{A}{K_{mA}}\right)^4 \tag{2}$$

$$r_{lysis} = \mu_{lysis}^0 \cdot X_d \tag{3}$$

After parameter estimation, the model successfully represents the experimental results in the range of the studied experimental conditions.

Acknowledgements
The authors are grateful to Spain's Ministry of Education and Science for funding this research (AGL2002-01712, PET2006_0827), and also to Grupo SOS, Spain for their invaluable help and information.

1. Cho, M.H. and Wang, S.S., ***Biotechnol. Bioeng***., **6**, 164-167 (1990).
2. Mori, A et al., ***J. Ferment. Technol***., **48**, 203-212 (1970).
3. Nanba, A. et al., ***J. Ferment. Technol***., **62**, 501-505 (1984).
4. Bar, R. et al., ***Biotechnol. Bioeng***., **29**, 796-798 (1987).
5. Park. Y.S. et al., ***Biotechnol. Bioeng***., **33**, 918-923 (1989).
6. Ito, T. et al., ***Appl. Microbiol. Biotechnol***., **12**, 295-299 (1991).
7. Romero, L.E. et al., ***Chem. Eng. J***., **54**, B15-B24 (1994).
8. Kruppa, R.K. et al., ***Bioprocess. Eng***., **20**, 545-551 (1999).
9. González-Sáiz, J.M. et al., ***Biotechnol. Prog***., **19**, 599-611 (2003).

PIII-6: Influence of Loading Rate and Air-flow Rate on Free Amino acid, Urea and Ammonium Ion Contents for Submerged Wine Vinegar Production

J.C. Mauricio[1], I.M. Santos-Dueñas[2], T. García-Martínez[1], M.C. Millán[1], J. E. Jiménez-Hornero[3], I. García-García[2]

[1]*Microbiology Department,* [2]*Chemical Engineering Department,* [3]*Computing and Numerical Analysis Department, University of Cordoba, PO Box 14071 Cordoba, Spain.*
E-mail: mi1gamaj@uco.es

Wine vinegar is mainly obtained by using a semi-continuous process involving submerged acetic acid bacteria. This operational procedure yields high productivities and affords easier control of some variables including the ethanol concentration at unloading time, unloaded volume, loading rate and air-flow rate. Such variables influence the concentration and activity of acetic acid bacteria as they act simultaneously on the acidity, ethanol concentration, oxygen supply and even temperature of the medium. In the scope of a study aimed to optimize the process, influence of these variables on biochemical and microbiological aspects must be considered.

This paper reports the results of a study of the influence of the loading rate and air-flow rate on the free amino acids, urea and ammonium ion concentrations in the broth. These compounds are important as nitrogen source for bacteria, can influence vinegar aroma (quality) and, some, can be precursors of harmful compounds[1].

A Montilla-Moriles vinegar, new Spanish protected origin denomination, has been produced. Experiments were conducted in a Frings 8 L reactor using the following operational procedure: depletion of ethanol in the medium to a concentration of 0.5 % (v/v) at a constant temperature of 31 ºC and an also constant air flow-rate of either 60 L h^{-1} or 30 L h^{-1}. Once the desired ethanol concentration was reached, 75% of the tank content was unloaded. Finally, the tank was loaded in a continuous way at a constant rate of either 0.01 L min^{-1} or 0.06 L/min^{-1}.

The cycle duration, mean acetification rate, acetic acid productivity and concentration of bacteria were not substantially affected by the higher loading rate. Nevertheless, it seems to cause a higher stress on the acetic acid bacteria, which involve an increase in the consumption of nitrogen source being L-proline, ammonium ion and L-methionine the most depleted. In contrast, the lower loading rate entails a substantial increase in the urea concentration.

As expected, the aeration rate had an important effect on the overall process rate[2]. The lower aeration flow rate slows down the fermentation and produces an increase in the consumption of assimilable nitrogen, probably as consequence of the higher duration of the cycle. In this case, L-proline continues being the most consumed nitrogen source. Finally, higher ammonium ion consumption was observed when a lower aeration rate was used.

Acknowledgements
The authors are grateful to Spain's Ministry of Education and Science for funding this research (AGL2002-01712, PET2006_0827), and also to Grupo SOS, Spain for their invaluable help and information.

1. Valero, E. et al., ***J. Sci. Food Agric.***, **85**, 603-608 (2005)
2. Rubio-Fernández, H. et al., ***Eur. Food Res. Technol.***, **219**, 393-397 (2004)

PIII-7: Free Amino acids, Urea and Ammonium Ion Composition of Vinegar from Biologically Aged Wine

J.C. Mauricio[1], I.M. Santos-Dueñas[2], T. García-Martínez[1], M.C. Millán-Pérez[1], J. E. Jiménez-Hornero[3], I. García-García[2]

[1]*Microbiology Department,* [2]*Chemical Engineering Department,* [3]*Computing and Numerical Analysis Department, University of Cordoba, PO Box 14071 Cordoba, Spain.*
E-mail: mi1gamaj@uco.es

Nitrogen source can be a limiting factor for acetic acid bacteria in the production of wine vinegar; this problem may worsens in wines subjected to biological ageing as sherry wine type. In this case, young wines undergo a second fermentation by flor yeasts[1]. So, biologically aged wines have a lower total content in assimilable nitrogen compounds than young wines[2]; additionally, a different profile composition use to be found too.

This work compares the composition in free amino acids, urea and ammonium ion between two different vinegars: one produced from white young wine and other one from a previously aged wine. Both wines are from the Montilla-Moriles region (Spain).

Experiments were conducted in a Frings 8 L reactor using the following operational procedure: depletion of ethanol in the medium to a concentration of 0.5 % (v/v) at a constant temperature of 31 ºC and an also constant air flow-rate of 60 L h^{-1} . Once the desired ethanol concentration was reached, 75% of the tank content was unloaded. Finally, the tank was loaded in a continuous manner at a constant rate of 0.06 L/min^{-1}.

A comparison between these wines shows that the aged one had a lower concentration in total assimilable nitrogen. Flor yeasts have completely consumed L-methionine, L-ornithine, α-aminobutyric acid and L-arginine, as well as, part of ammonium ion and L-leucine. On the other hand, the concentrations of urea, L-aspartic acid, L-serine and L-proline have slightly increased.

The consumption of total assimilable nitrogen source during the acetification process was about 50% in both cases, being L-proline and L-leucine the amino acids preferably consumed. Nevertheless, some differences are observed, whereas young wine vinegar used L-metionine and L-ornitine, the production of aged wine vinegar used completely ammonium ion, urea, L-serine and other amino acids in minor proportion.

The results suggest that nitrogen source is not a limiting factor when an aged wine is used for vinegar production. In addition, it is highlighted the great adaptability of acetic acid bacteria for using the available nitrogen sources in every conditions.

Acknowledgements
The authors are grateful to Spain's Ministry of Education and Science for funding this research (AGL2002-01712, PET2006_0827), and also to Grupo SOS, Spain for their invaluable help and information.

1. Berlanga, T.M. et al., ***J. Agric. Food Chem.***, **52**, 2577-2581 (2004)
2. Mauricio, J.C. and Ortega, J.M., ***Biotechnol. Bioeng.***, **53**, 159-167 (1997).