Modelling of Acetic Acid Biosynthesis at Low Acid Concentration

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An experimental investigation concerning the acetic acid formation in a fixed packed bed reactor was carried out at low acid concentration. The influence of air flow rate and ethanol initial concentration was investigated. For low concentration of acetic acid in the reaction medium two models were proposed. In these models interphase oxygen transfer or biochemical reaction is considered to determine the overall process reaction rate. A comparison between theoretical and experimental data was done.

Keywords: acetic acid, mathematical models, low concentration

Acetic acid is an important chemical species with multiple applications in chemical and food industries. In many industrial processes, it is used as solvent or to prepare other valuable products such as acetic esters. Acetic acid can be obtained by synthetic route or by fermentation. Fermentation is an ideal way to produce carboxylic acids because it uses renewable resources.

The state of art in industrial vinegar production is a batch based aerobic bioprocess with a partial renewal of the fermentation broth at the end of each cycle. As microorganisms, *Acetobacter* are used to produce acetic acid from alcohol. Vinegar fermentation is essentially a two-step process comprising the anaerobic conversion of sugars to ethanol and the aerobic oxidation of ethanol to acetic acid [1]. The rate of oxygen transfer to the cells is one of the limiting factors to obtain acetic acid. It is known that increasing of oxygen rate transfer can enhance the rate of product formation.

One of the frequently used reactors in acetic acid production is trickle-flow reactor in which microorganisms are attached to the inert material (particles) as a biological film. These reactors assure a large area for gas-liquid transfer [2]. For this fermenter, where a high level of oxygen in the reaction medium is assured, the inoculums is directly distributed or mixed in the recycled reaction media of the packed tower [3, 4].

This paper presents an experimental study concerning acetic acid fermentation in a trickle flow reactor in order to develop adequate mathematical models that describe the process dynamics. Using experimental data from a bench scale reactor, two simplified models are validated, one for the case in which oxygen transfer from air to the fermentation medium is determinant for the process and the second where the process can be characterized, for the ethanol consumption, with Monod kinetics.

Mathematical Modelling

A simplified scheme of experimental set-up is presented in figure 1. The biochemical reaction occurs at the solid - liquid film interface. A diffusion layer surrounds the biofilm attached to the inert particle (wood) surface. Oxygen from air diffuses towards reaction surface over the gaseous and liquid films. Reaction volume can be considered as liquid volume from the fixed bed, because here, the immobilized bacteria are in contact with culture media (ethanol and liquid dissolved oxygen). At low acetic acid concentration, the process can be controlled by interfacial oxygen transport or by substrate consumption (biochemical reaction). A specific mathematical model will characterize each case.

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The first model presumes that *oxygen transport is a determinant step for the process*.

The oxygen flow rate towards wood surface, where biochemical reaction occurs, is given by (1):

$$n_O = K \cdot A_u \cdot \Delta C_{O,m} = K \cdot \frac{6}{d_p} \cdot Z_u \cdot S \cdot \left(\frac{y_{O,m} \cdot p}{H} \cdot \frac{\rho_l}{M_l} \right) \quad (1)$$

Acetic acid mass balance with respect to the packed bed reactor can be written:

$$\varepsilon_l \cdot Z_u \cdot S \cdot \frac{dc_{A2}}{d\tau} = L_v \cdot c_{A1} - L_v \cdot c_{A2} + 2 \cdot n_O \quad (2)$$

From relations (1) and (2) and considering reaction stoechiometry one may write:

$$\frac{dc_{A2}}{d\tau} = \frac{L_v}{\varepsilon_l \cdot Z_u \cdot S} \cdot (c_{A1} - c_{A2}) + K \cdot \frac{12}{d_p \cdot \varepsilon_l} \cdot \frac{y_{O,m} \cdot p}{H} \cdot \frac{\rho_l}{M_l} \quad (3)$$

Acetic acid mass balance with respect to recycling vessel, where the reaction can be neglected as a consequence of oxygen lack and of short residence time, is given by eq. (4):

$$\frac{dc_{A1}}{d\tau} = \frac{L_v}{V - \varepsilon_l \cdot Z_u \cdot S} \cdot (c_{A2} - c_{A1}) \quad (4)$$

Because Henry's equilibrium constant for oxygen absorption depends on ethanol and acetic acid concentrations in the liquid phase the following relation was used [5]:

$$H = H_0 \cdot \left(\frac{c_{E,0}}{c_E} \right)^{\alpha} \cdot \left(1 - \beta \cdot \left(\frac{c_{A1}}{c_{A\max}} \right)^{\gamma} \right) \quad (5)$$

The model equations can be solved with the initial conditions:

$$\tau = 0, \quad c_{A1} = c_{A2} = c_{Ao} \quad (6)$$

The second model accepts that the *substrate consumption (biochemical reaction) is a determinant step for the process rate*. For this case, the substrate consumption rate is specified by Monod type kinetics:

(7)

The acid flow rate can be expressed as follows:

$$n_A = v_{R,A} \cdot \varepsilon_l \cdot Z_u \cdot S \quad (8)$$

Substituting alcohol concentration and the expression for substrate consumption rate arrived to the following:

$$n_A = \frac{v_{R,\max} \cdot \left(c_{E,0} - \left(c_{A2} - c_{A20} \right) \right)}{K_S + \left(c_{E,0} - \left(c_{A2} - c_{A20} \right) \right)} \cdot \varepsilon_l \cdot Z_u \cdot S \quad (9)$$

The acid mass balance equation for a local control volume of the reactor is given by:

$$\varepsilon_l \cdot Z_u \cdot S \cdot \frac{dc_{A2}}{d\tau} = L_v \cdot c_{A1} - L_v \cdot c_{A2} + n_A \qquad (10)$$

Finally, substrate concentration at the reactor exit is given by:

$$\frac{dc_{A2}}{d\tau} = \frac{L_v}{\varepsilon_l \cdot Z_u \cdot S} \cdot (c_{A1} - c_{A2}) + \frac{v_{R,\max} \cdot (c_{E,0} - (c_{A2} - c_{A20}))}{K_S + (c_{E,0} - (c_{A2} - c_{A20}))} \qquad (11)$$

Acetic acid mass balance equation for the recycling vessel without oxygen in liquid phase may be expressed by eq. (4). For v_{rmax} the following expression is proposed [6-8]:

$$v_{R,\max} = \frac{v_{R,\max 0} \cdot c_{mO}}{Y_{S,mO}} = v_{R,\max 0} \cdot \left(1 + \delta \cdot \left(\frac{K}{K_f}\right)^{\varepsilon}\right) \qquad (12)$$

Initial conditions for this model are:

$$\tau = 0, \qquad c_{A1} = c_{A2} = c_{A20} = c_{A0} \qquad (13)$$

Experimental part

A schematic flow sheet of the experimental system is presented in figure 1 and was widely described in previous paper [9]. This device assures an important inter-phase contact surface. The packed bed was sterilized in an autoclave (121^{0}C, 20 min). As carbon source, an alcoholic solution, resulted from molasses fermentation with no more than 200 g/L glucose equivalent, was used. As inoculum *Acetobacter* strain was used from acidified wine. The culture medium consisted of glucose (10 g), peptone (10 g), glycerin (25 cm^3) and vinegar (100 cm^3, with minimum 20 g/L acetic acid). Inoculum was incubated at 30^{o}C, under slow agitation for 24 h and than was aseptically added in bioreactor at the beginning of aeration. Temperature and *p*H were monitored. After minimum 48 hours, fermentation was stopped. Samples were collected aseptically for establishing the acid content, by titration with standard NaOH 0.1N solution in presence of phenolphthalein.

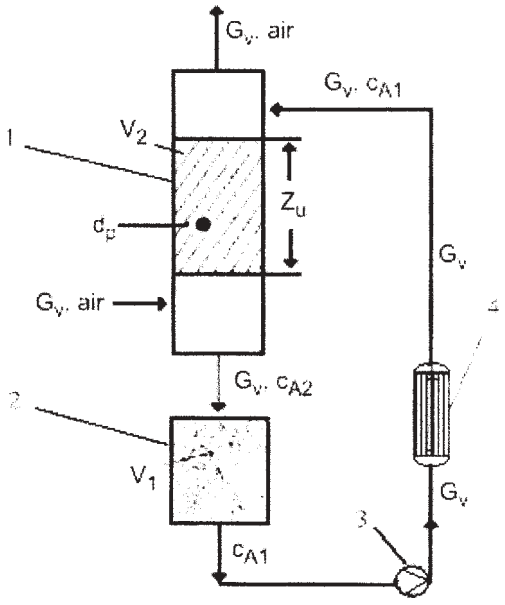

Fig. 1. Laboratory fermentation experimental set-up 1-fixed bed reactor, 2-recycling vessel, 3-recycling pump, 4- heat exchanger

Results and discussions

In order to apply the models to experimental data, the models' parameters must be estimated (some of parameters were experimentally established elsewhere [5]). For both models column diameter (d=0.05 m), packed elements diameter (d_p=0.01 m), packed bed height (Z_u=0.3 m), column liquid volume (V=0.75·10^{-3} m^3) were the same. Also, the same liquid rate was used L_v = 2.083 ·10^{-6} m^{3}s^{-1}. Oxygen mass transfer coefficient K was estimated from the following relation: $Sh=0.015Re^{0.66}Sc^{0.33}$, in which characteristic length is packed elements diameter, d_p. Liquid fraction was estimated from the following relation $\varepsilon_l=0.02+0.15q_{vl}$, where q_{vl} is specific liquid flow rate.

The first model needs the evaluation of four parameters. The values proposed for these parameters are: H_0=6200·10^5 N m^{-2}, α=0.33, β=0.1 and γ=1.

The second model has more parameters. For the second model, the proposed values of some parameters are: K_S=250·10^3 mol m^{-3}, $v_{R,maxo}$ = 650 mol m^{-3}s^{-1}, δ=0.5, ε=0.66. Oxygen mass transfer coefficient at bed flooding K_f can be determined from the following relation $Sh=0.015Re_f^{0.66}Sc^{0.33}$, the characteristic length being packed elements diameter, d_p.

The models equations were solved using a fourth-order Runge-Kutta algorithm. The models' test of adequacy in describing the formation of acetic acid is done by comparison of the models predictions with experimental data. The ability of the models to predict the experimental behavior is analyzed for two experiments and is presented in figures 2 and 3.

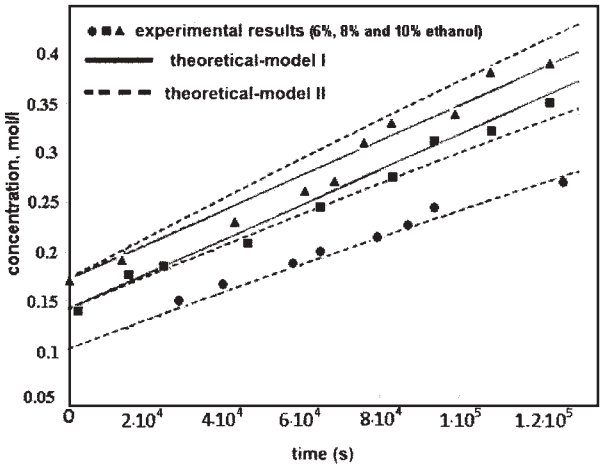

Fig. 2. Experimental and calculated acetic acid concentration vs. time for different initial ethanol concentrations at L_v=2.083·10^{-6} m^{3}s^{-1} and G_v=9.72·10^{-5} Nm^3 s^{-1}

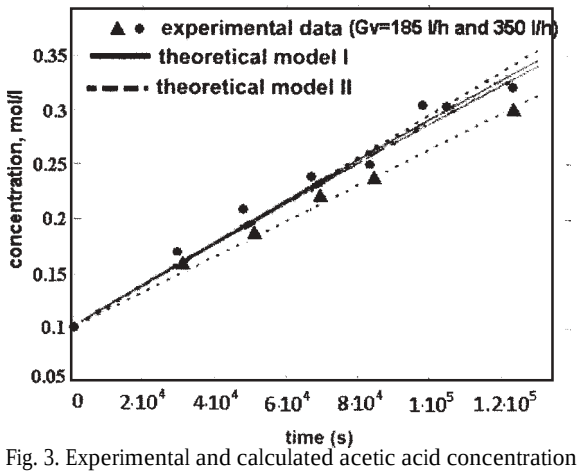

Fig. 3. Experimental and calculated acetic acid concentration vs. time for different air flow rates, at L_v=2.083·10^{-6} m^{3}s^{-1}, and initial ethanol concentration $c_{E,0}$=10% (mass percent)

Figure 2 shows a comparison between experiment and theory in the case in which ethanol concentration varied from 6% to 10% (mass percent) in the feeding rate. Air flow rate was $G_v = 9.72 \cdot 10^{-5}$ Nm^3 s^{-1}. The continuous lines represent the theoretical acetic acid concentration predicted by the first model, and the dashed curves represent the theoretical prediction for the second model. As it can be seen for a lower ethanol concentration, the both models predict the same theoretical curves. In the case of higher ethanol concentration, the theoretical model that considers the oxygen consumption rate to be determinant for the process is more adequate.

Figure 3 shows a comparison between experiment and theory in the case in which two different air flow rate are used in experiment: $G_v = 5.14 \cdot 10^{-5}$ Nm^3 s^{-1} and $9.72 \cdot 10^{-5}$ Nm^3 s^{-1}. Initial ethanol concentration used in experiment was $c_{E0} = 10\%$ (mass percent). In this case, the second model is more adequate. An explanation could be that in this case, when the varied parameter is the air flow rate, the first model has a low sensitivity.

Conclusions

In the present study the influence of ethanol concentrations and air flow rate for acetic acid production was investigated in order to validate the two proposed models.

First mathematical model presumes that acetic acid dynamics in the reaction medium is controlled by oxygen transport from air to liquid phase. The second model is based on the consideration that acetic acid dynamics in the reaction medium is influenced by substrate consumption rate. The advantage of the first model is that it operates only with mass transfer considerations and is not necessary to presume a kinetic reaction. The second model uses Monod kinetics, which is more common for biological substrate uptake. A good agreement between experiment and theoretical predictions was obtained using the two models.

Symbols

A_u - packed bed surface, m^2
c_{A0} - initial acetic acid concentration, mol m^{-3}
c_{Al} - acetic acid concentration of the bioreactor feed, mol m^{-3}
c_{A2} - acetic acid concentration (bioreactor out), mol m^{-3}
c_{A20} - initial acetic acid concentration (bioreactor out), mol m^{-3}
c_{Amax} - theoretical acetic acid concentration, mol m^{-3}
$c_{E,0}$ - initial ethanol concentration , mol m^{-3}
c_E - ethanol concentration, mol m^{-3}
$c_{O,m}$ - mean oxygen concentration at the bacteria level, mol m^{-3}
$c_{O,s}$ - oxygen constant quantity on the packed bed surface, mol m^{-3}
d - packed bed column diameter, m
d_p packed elements diameter, m
G_v - air flow rate, Nm^3 s^{-1}
L_V - liquid flow rate, m^3 s^{-1}
H- Henry's constant of the biochemical reaction medium, N m^{-2}
H_0 - Henry Constant for O_2 absorption in standard molasses solution, N m^{-2}
K - oxygen mass transfer coefficient, m s^{-1}
K_f - oxygen mass transfer coefficient at bed flooding, m s^{-1}
K_S - Monod reaction constant, mol m^{-3}
M_l - liquid mean molar mass, kg mol^{-1}
n_A - acid acetic flow rate, mol l^{-1} s^{-1}
n_O - oxygen molar flow rate, mol l^{-1} s^{-1}
p - total pressure, N m^{-2}
$p_{O,m}$ - oxygen partial pressure in gaseous phase, N m^{-2}
q_{vl} - liquid specific flow rate, mol l^{-1} s^{-1}
Re- Reynolds number for liquid flow in fixed bed
Re_f - Reynolds number for liquid flow in a flooded fixed bed
S - column section, m^2
Sc - Schmidt number for oxygen transfer in molasses solution
$\nu_{R,max}$ -maximum reaction rate, mol m^{-3}s^{-1}
$\nu_{R,max0}$ - Aparent Monod reaction constant, s^{-1}
$\nu_{R,A}$ -acetic acid formation rate, mol m^{-3}s^{-1}
$\Delta C_{O,m}$ - mean oxygen concentration,mol m^{-3}
V - column liquid volume, m^3
$y_{O,m}$ - oxygen molar concentration in the gaseous phase, mol/mol
$Y_{S,mO}$ - macroscopic specific yield of oxygen, mol kg^{-1}
Z_u - packed bed height, m

Greek Letters

α - dimensionless parameter in relation (5)
β - dimensionless parameter in relation (5)
γ - dimensionless parameter in relation (5)
δ - dimensionless parameter in relation (12)
ε - dimensionless parameter in relation (12)
ε_l - liquid fraction, m^3 m^{-3}
p_l - liquid mean density, kg m^{-3}
τ - time, s

References

1. HORIUCHI, J., TABATA, K., KANNO, T., KOBAYASHI, M., Continuous Acetic Acid Production by a Packed Bed Bioreactor Employing Charcoal Pellets Derived from Waste Mushroom Medium, J. Biosci. Bioeng., **89:126**, 2000, p. 30
2. MOULIJN, J.A., MAKKEE, M., VAN DIEPEN, A., Chemical Process Technology, Wiley, New York, 2001
3. LOPEZ, A., JOHNSON, L.W., WOOD, C.B., Observation on a Laboratory for Submerged Acetic Fermentation, Applm Microbiol., **9**, nr. 5, 1961, p. 425
4. ROMERO, L.E., CANTERO, D., Mathematical Model for Liquid-Gas Equilibrium in Acetic Acid Fermentations, Inc. Biotechnol. Bioeng., **59**, 1998, p. 310
5. DOBRE T., FLOAREA O., BABÃTÃ, M., Gas-Liquid Mass Transfer in Rotating Disk Contactor, Proceedings of the X Romanian International Conference on Chemistry and Chemical Engineering, **1**, 1997, p. 46
6. CELINE, P.B., BOHATIER, C.L., GHOMMIDH, C.H., Modelling the Kinetics of Growth of Acetic Bacteria to Increase Vinegar Production: Analogy with Mechanical Modelling, http:// www. univ-perp.fr/ mtns2000/articles/B57.pdf
7. YAMADA,Y., KAWASE,Y., Aerobic Composting of Waste Activated Sludge: Kinetic Analysis for Microbiological Reaction and Oxygen Consumption, Waste Management, **26**, 2000, p. 49
8. TOKSAY ONER, E., KYRDAR, B., ONSAN, Z., ULGEN, K., Modelling of the Induced Expression for High Level Production of a Foreign Protein by Recombinant E.coli under the Control of the T7 Phage Promoter, Process Biochemistry, **39**, 2003, p. 315
9. STROESCU, M., DOBRE, T. OFIÞERU T., Some Aspects Concerning Transformation of Molasses Alcoholic Fermentates in Proceedings of the XIVth Romanian International Conference on Chemistry and Chemical Engineering, **3**, 2005, p. 318

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