

Characterisation of Gas Mixed Bioreactors in Submerged Citric Acid Fermentation

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In memoriam Prof. Emeritus Vera Johanides

Bioprocess quantities of submerged citric acid fermentation on beet molasses substrate in 50 l Bubble Column (*BCR*) and External Loop Reactor (*ELR*), were studied. Mixing properties, volumetric oxygen transfer coefficient and their influence in both bioreactors, were studied. For the sake of comparison some measurements were carried out in STR with radial impeller Rusthon turbine, as well as Effigas, the axial one. Comparing the results of *BCR* and *ELR*, it is evident that *ELR* enables the development of productive morphology and more homogenous biomass distribution than *BCR*.

Key words:

Citric acid, bubble column, external loop reactor, hydrodynamic behavior

Introduction

In general, stirred tank reactors are still the most widely applied type of bioreactors also in citric acid production.¹ This type of reactor offers a few advantages, such as the ease with which the residence of gas phase, intensity of agitation, mass and heat transfer can be varied. The main disadvantages of this reactors are high energy consumption and high maintenance costs.

On the contrary, gas mixed bioreactors represent simple design and operation, low energy input and maintenance costs. This type of reactors are very suitable, especially, in those processes expressing Newtonian behavior of fermentation broth. External or internal, air lift reactors were applied in various bioprocesses including bacterial, yeast and tissue cultures. Although filamentous fungi broths often show pseudoplastic, non Newtonian character, and its great influence on reduction of hydrodynamic conditions in bioreactor, gas mixed bioreactors, were successfully applied in several cases.^{2,3}

An appropriate bioreactor design, could besides all other factors significantly influence on oxygen transfer, process rheology and the morphology of the productive organism.^{3,4} Several factors such as temperature, pressure, use of pure oxygen or mixtures oxygen-air, power input, aeration or an appropriate bioreactor design could effectively influence the oxygen transfer, the process rheology and the morphology of the productive organism.^{5,6,7}

A high oxygen concentration and increased respiration rate demands, in the period of exponential growth phase, a higher oxygen input. High citric acid production rate is related to dissolved oxygen concentrations from 5.0 to 7.0 mg L⁻¹ O₂ in substrate.³ Oxygen concentrations in bioreactor could vary between critical concentration 3.0 mg L⁻¹ O₂ up to 10.0 mg L⁻¹ O₂. Higher concentrations than 10.0 mg L⁻¹ O₂, influence the changes in *Aspergillus niger* morphology, promoting formation of unproductive "blown cells" and therefore reducing the biosynthesis of citric acid.⁴ This is often a problem in processes where for increasing the oxygen mass transfer a mixture of pure oxygen and air is used.⁸

The objectives of this work were related examination of the abilities of gas mixed bioreactors, as are *BCR* and *ELR*, and the influence of their performances, as mixing properties and volumetric oxygen transfer, on citric acid production.

Experimental

Microorganism

An *Aspergillus niger* strain A60 (NRRL 2270) was used in all experiments. Conidia taken from 7 day old cultures on worth agar slants incubated at $T = 30\text{ }^{\circ}\text{C}$ were used as inocula for the fermentation process. The initial spore concentration of the inoculum was $5 \cdot 10^7$ conidia mL⁻¹ as determined spectroscopically.⁹ The growth of mycelia was followed by optical microscopic observations (Wild M-20, Switzerland)

Media composition

The fermentation substrate was diluted beet molasses with total of 12.5 % reducing sugars. It was treated by addition of potassium hexacyanoferrate $K_4[Fe(CN)_6]$, which balanced the ratio of heavy metals ions by the formation of metal complexes.¹⁰ The optimal addition of $K_4[Fe(CN)_6]$, H_3PO_4 and pH was determined for every molasses batch in shaken flask experiments. $K_4[Fe(CN)_6]$ was added in two stages, before sterilization (primary additive) and after (secondary additive).¹¹

Bioreactors

Geometric ratio D/H in *BCR* and *ELR* was 1 : 20. Diameter of *BCR* and *ELR* riser was $D = 0.15$ m and diameter of external loop was $D_R = 0.05$ m. As aerator in both reactors a glass sintered plate of porosity $30 \mu\text{m}$ and 10 mm thickness was used. All experiments were performed at aeration flow rate $Q_g = 1.0 \text{ L s}^{-1} \text{ L}^{-1}$ substrate and process temperature $T = 30 \text{ }^\circ\text{C}$.

Stirred tank reactor (*STR*) experiments were performed in 10 Chemap SG 3000 reactor (Chemap AG Switzerland). Diameter of *STR* was $D = 0.25$ m and diameter of *Rushton* radial impeller and *Effligas* (Chemap AG, Switzerland) axial turbine were $D_R = 0.08$ m. All experiments were performed at aeration flow rate $Q_g = 1.0 \text{ L s}^{-1} \text{ L}^{-1}$ substrate and process temperature $T = 30 \text{ }^\circ\text{C}$.

All reactors were equipped with sterilizable Ingold pH, redox electrodes, and Industrial Lab pO_2 electrode, automatic foam control and temperature control unit. For *on-line* measurements an Intec pH meter M-7822N and redox meter 005-300000, were used. Oxygen partial pressure measurement was measured by a polarographic electrode, Industrial Lab MFG 509 with IL amplifier Type 531.

Experimental methods

Volumetric oxygen transport coefficient in fermentation broth was measured by dynamic method^{8,12} on sampling points H_1 and H_2 (Fig.1), using polarographic electrodes Industrial Lab MFG 509 with IL amplifier Type 531. Correction of measurements were made according to electrode response time, gas and liquid dynamics according to *Dang et al.* 1977.¹³

For the measurements of the mixing time conductometric method was used.¹⁴ Two stainless steel electrodes ($d = 8 \cdot 10^{-3}$ m, $l = 400 \cdot 10^{-3}$ m at distance $d_c = 5 \cdot 10^{-3}$ m) on sampling points H_1 and H_2 (Fig. 1), in connection with conductometer, Radiometer Copenhagen Type CMD 2 D, with and recorder BBC Goerz Servogor 311 were used. As a tracer electrolyte 500 ml, 1 mol L^{-1} Na Cl was used.

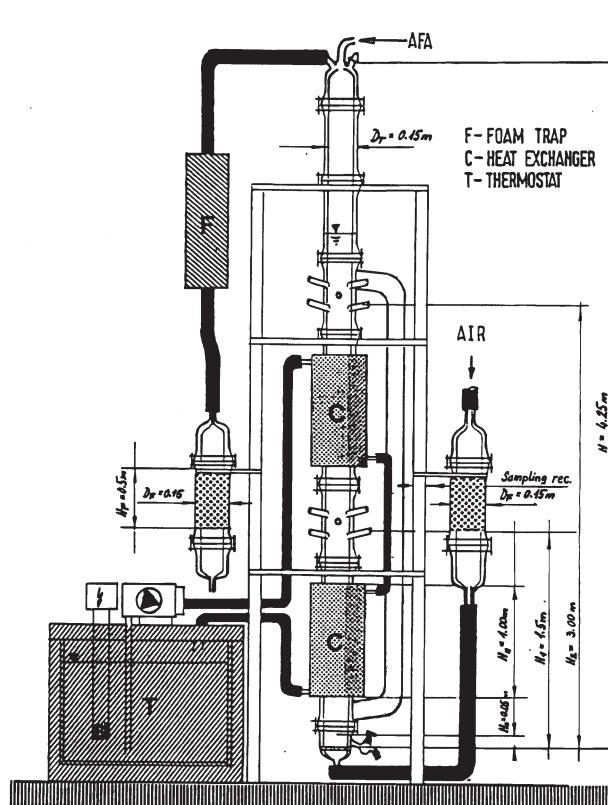


Fig. 1 – Experimental setup of 50 l Bubble Column and External Loop Reactor

Rheological measurements of flow behavior and fluid consistency index, were performed using a Rheotest 3 viscometer in a double cylinder configuration (2VEB MLW, Pruferte-Werk, Medingen, Germany). A rotating cylinder ($d = 37.7$ mm inside diameter) and measuring cell (D040.39 mm outside diameter) were used. The rheological properties were characterized by the Ostwald de Waele power law model.⁸

Biomass was determined gravimetrically after drying at $105 \text{ }^\circ\text{C}$ on constant mass. Citric acid and sugar content were determined by HPLC using Aminex HPX- 87 H Column $300 \cdot 7,8$.^{4,9}

All of results were summarised and presented in Figs. 2–5 and 7–9, as the results of typical batch fermentation courses. A decrease of the fermentation broth volume caused by evaporation and sampling was taken in to account in all experiments.

Experimental results and discussion

Citric acid fermentation broth is rheologically characterized by biomass concentration and its physiology state. While the first part from the inoculation up to 18 hours represents a typical Newtonian behavior, in the second part from 18 to 168 hours parallel increasing of the biomass and

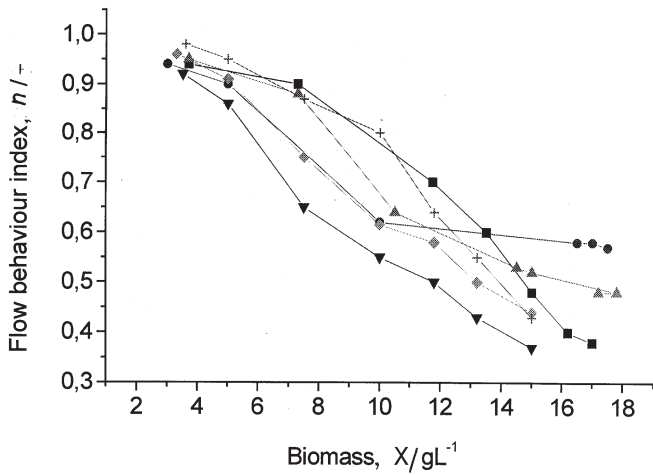


Fig 2 – The changes of the flow behavior index n with biomass concentration X

■ ELR riser + ELR recycle
 ◇ STR radial mixing ● STR axial mixing – Effigas
 △ BCR H_1 , ▼ BCR H_2

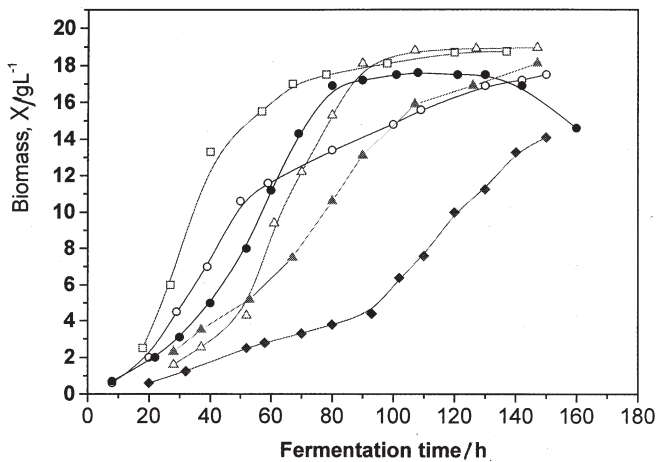


Fig 3 – The time course of biomass concentration X

△ ELR riser ▲ ELR recycle
 ● STR radial mixing □ STR axial mixing – Effigas
 ◆ BCR H_1 , ○ BCR H_2

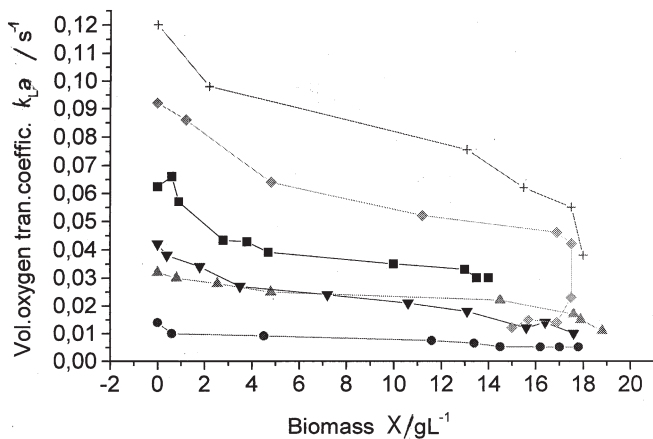


Fig 4 – Volumetric oxygen transfer coefficient k_La as a function of biomass concentration X

△ ELR riser ▼ ELR recycle
 ◇ STR radial mixing + STR axial mixing – Effigas
 ■ BCR H_1 , ● BCR H_2

pseudoplastic behavior of the fermentation broth, was detected ^{6,7}.

This quality is influenced by microbial morphology, as well as by production of fungal polysaccharides produced by young hyphae tips as a sticky substance needed for fixation of fungal hyphae on solid matrix in the nature.⁸

During a citric acid production stage polysaccharides and lysis product are released in fermentation substrate largely contributing the decreasing of flow behavior coefficient n . In the range of biomass concentration up to 3.0 g L^{-1} , flow behavior index is $n = 1.00 - 0.95$, while in further growth of biomass from 3.0 to 17.5 g L^{-1} it decreases to $n = 0.43$ at increasing of fluid consistency index K up to 0.135 Pa s^n

In Fig. 2, the changes in flow behavior index n influenced by the increasing of biomass concentration, are presented. The most relevant and fast change is indicated in BCR in the upper part of the column at the sampling point H_2 (Fig. 1), where microbial pellets with long and thin peripheral hyphae and highest biomass concentration, were detected. This low citric acid production form expressed significant resistance to the flow pattern in this part of the BCR.

In the lower part of BCR at sampling point H_1 (Fig. 1), rheological behavior of the fermentation broth was related to low biomass concentration (Fig. 3), as well as its small, thick, and short hyphae peripheral pellets which remains on morphological forms from STR or ELR.

By increasing biomass and pseudoplasticity in fermentation broth k_La coefficient is obviously reduced. Lower part of BCR at H_1 is able to compare with STR, while in the upper part at H_2 low oxygen transfer, was observed (Fig. 4).

Mixing abilities in BCR are characterized by decreasing of mixing time in the upper part of BCR at H_2 , while in the lower region at H_1 (Fig. 1), significant prolongation of mixing time with increased superficial gas velocity, was indicated (Fig. 5).

Heterogeneous regions are related with very bad back mixing in BCR. (Fig. 6). The velocity profile of the liquid phase, the non uniform distribution of the gas hold-up across the column cross section, and the individual rising velocities of bubbles of different size, resulted in formation of three zones with different two phase structure.¹⁵

In central region zone (A), in the up flow region, there exists a wide bubble size distribution and heterogeneous two phase structure, as a result of preferential bubble coalescence in the center of the column. In back flow zone (B) accumulate small bubbles with relatively long residence times. The area of this zone depends on the intensity of

aeration, but does not exceed more than one third part of the *BCR* height. In region above the gas distributor, in zone (C), the back mixing is practically nil.

The differences in biomass concentration and morphology in *BCR* can be also induced by heterogeneous two phase structure and the differences in oxygen concentration in various area.

The experimental results show that bubble column reactor could be described as a two compartment model bioreactor. The lower part *BCR* H_1 (Fig. 1) was indicated as the area of high $k_L a$ influenced by small bubble size, high specific interfacial area a and high hydrostatic pressure, in opposite with the upper part *BCR* at H_2 (Fig. 4). Different concentration of fungal biomass in both compartment are reflecting different specific respiration rates. The difference in their maxims at 40 hours, ($0.038 \text{ mg L}^{-1} \text{ s}^{-1} \text{ O}_2$) at H_1 and ($0.110 \text{ mg L}^{-1} \text{ s}^{-1} \text{ O}_2$) at H_2 (Fig. 7) also confirms the heterogeneity of growth conditions in both compartment. ⁷

The results of fermentations in *BCR* and *ELR* in comparison with axially and radially mixed STR (Figs. 2,3,4,7,8), showed that for filamentous fungi fermentation and its pseudoplastic behavior broth, *BCR* is not the most suitable reactor. Comparing the efficiency of axial and radial mixing in Stirred Tank Reactor, it was found that in axial mixing system high shear field of the *Effigis* turbine did not destroy *Aspergillus niger* pellets. In contrary, it influences the development of more uniform short and thick peripheral hyphae pellets, which enables high product yielding fermentations.

The morphology in the lower region H_1 of *BCR* (Fig. 1), is similar to the morphology in *STR*. It seems that a higher shear field and higher oxygen concentration in the area close to aerator inhibits formation of thin filamentous hyphae pellets with larger diameter, typical for upper region of *BCR*.

On the contrary, with *BCR* fluid dynamics in *ELR* are more intensive, and therefore fermentation conditions are more homogenous at the same superficial gas velocities, than those in *BCR*. External loop enables effective back mixing that was also indicated by non significant differences and more unique pellet morphology. Spherical pellets produced in *ELR* are of small diameter size ($d_p = 0.5 - 0.8 \cdot 10^{-3} \text{ m}$), remains on those in lower part of *BCR* ($d_p = 0.4 - 0.6 \cdot 10^{-3} \text{ m}$) and *STR* ($d_p = 0.3 - 0.5 \cdot 10^{-3} \text{ m}$), and they are also more homogeneously distributed in the whole bioreactor space. Mixing patterns in *ELR* are close to axial mixing in *STR*. There were also indicated higher production of citric acid in *ELR*, than in those in *BCR* (Fig. 8). For efficient operation of *ELR* higher superficial gas velocity in

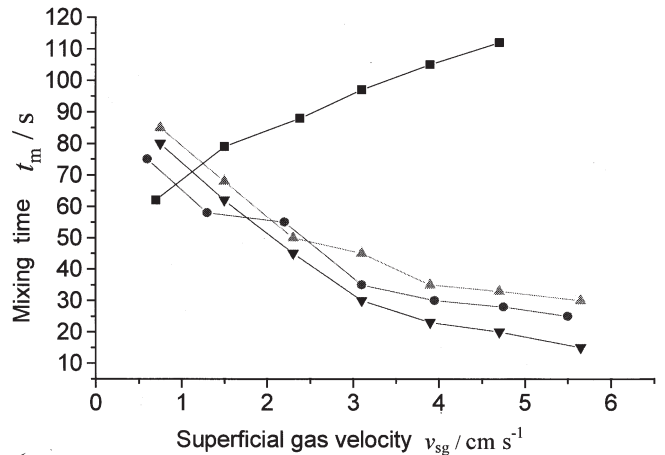


Fig. 5 – Mixing time t_m as a function of superficial gas velocity v_{sg}
 Δ ELR riser ∇ ELR recycle
 \blacksquare BCR H_1 \bullet BCR H_2

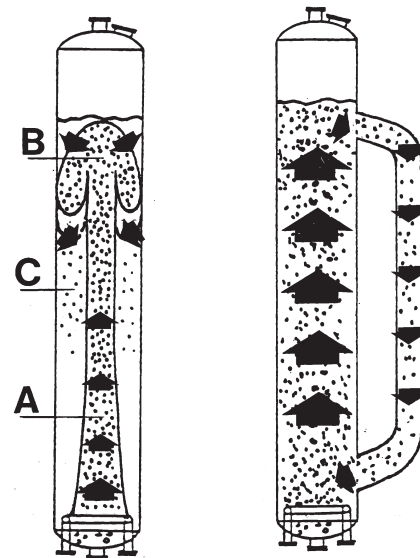


Fig. 6 – Heterogeneous two phase pattern in *BCR*

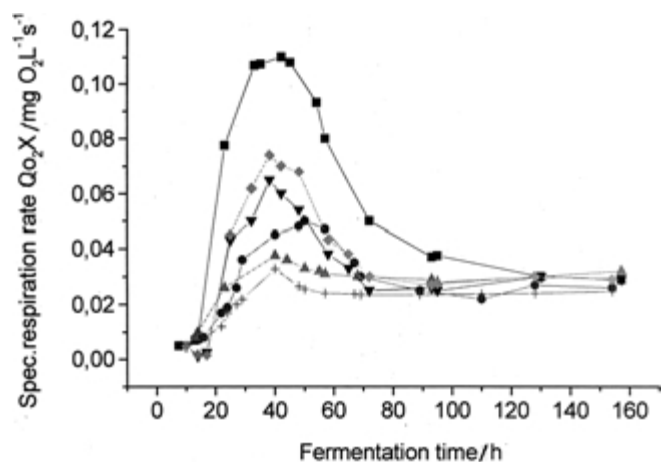


Fig. 7 – The time course of specific respiration rate $Q_{O_2} X$
 \blacklozenge ELR riser \blacktriangledown ELR recycle
 \bullet STR radial mixing + STR axial mixing – Effigis
 \triangle BCR H_1 \blacksquare BCR H_2

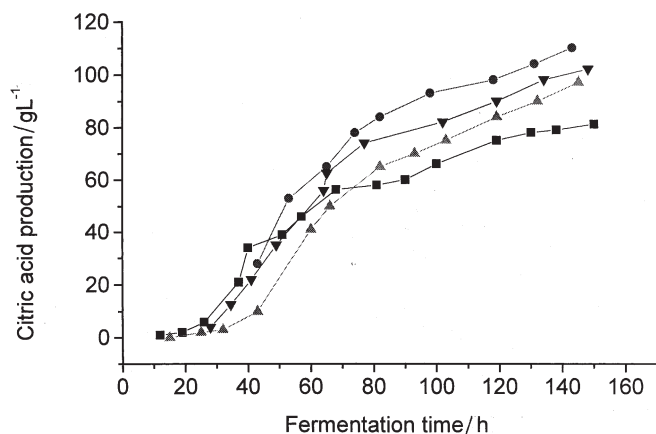


Fig. 8 – The time course of citric acid concentration P
 ▼ ELR riser ● STR radial mixing
 ■ BCR H_2 △ STR axial mixing – Effigas

the range up to $v_{sg} = 5.0 \cdot 10^{-2} \text{ m s}^{-1}$ and constant liquid level, are recommended.

ELR could be compared with axially mixed STR (Effigas) in H_1 and a STR in H_2 . Oxygen transfer coefficients in ELR are more uniform and a back mixing is more improved. Morphology of *Aspergillus niger* doesn't show such varieties and growth differences at various stages as in BCR. Although, volumetric oxygen transfer coefficient $k_L a$ in recycle is lower than in up-flow chamber, there the mixing and circulation time are higher. That influence of oxygen concentration deficiency on biomass in this part, was not observed.

Conclusions

When comparing bubble column and external loop bioreactors it can be concluded, that for fermentation broths with pseudoplastic rheology, typical behavior for fermentation by filamentous fungi growth, ELR would be more suitable bioreactor for citric acid fermentation. ELR enables the development of productive morphology and more homogeneous biomass distribution. At high superficial gas and liquid velocity rates it also spends less energy need for pneumatically mixing than mechanically mixed bioreactors.

The non-Newtonian properties of the whole fermentation broth depend entirely on the concentration and morphology of the biomass, since the liquid phase is a low viscosity Newtonian fluid. Presented data are rarely reported in the literature, probably, due to the industrial interests.

Symbols

a – specific interfacial area, m^{-1}
 D – diameter of bioreactor, m

d_p – diameter of microbial pellet, m

H, H_1, H_2 – height of bioreactor, height of sampling ports, m

K – fluid consistency index, Pas^n

$k_L a$ – volumetric oxygen transfer coefficient, h^{-1}

l – length, m

n – flow behavior index, –

P – citric acid concentration, g L^{-1}

$Q_{O_2 X}$ – specific respiration rate per unit volume, $\text{mg L}^{-1} \text{ s}^{-1} \text{ O}_2$

t_{mix} – mixing time, s^{-1}

v_{sg} – superficial gas velocity, m s^{-1}

X – biomass concentration, g L^{-1}

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