Microbial acetate oxidation in horizontal rotating tubular bioreactor

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The aim of this work was to investigate the possibility of conducting a continuous aerobic bioprocess in a horizontal rotating tubular bioreactor (HRTB). Aerobic oxidation of acetate by the action of a mixed microbial culture was chosen as a model process. The microbial culture was not only grown in a suspension but also in the form of a biofilm on the interior surface of HRTB. Efficiency of the bioprocess was monitored by determination of the acetate concentration and chemical oxygen demand (COD). While acetate inlet concentration and feeding rate influenced efficiency of acetate oxidation, the bioreactor rotation speed did not influence the bioprocess dynamics significantly. Gradients of acetate concentration and pH along HRTB were more pronounced at lower feeding rates. Volumetric load of acetate was proved to be the most significant parameter. High volumetric loads (above 2 g acetate l^{-1} h^{-1}) gave poor acetate oxidation efficiency (only 17 to 50%). When the volumetric load was in the range of 0·60–1·75 g acetate l^{-1} h^{-1}, acetate oxidation efficiency was 50–75%. At lower volumetric loads (0·14–0·58 g acetate l^{-1} h^{-1}), complete acetate consumption was achieved. On the basis of the obtained results, it can be concluded that HRTB is suitable for conducting aerobic continuous bioprocesses.

1. Introduction

Tubular bioreactors are suitable for both anaerobic and aerobic bioprocesses despite the common belief that they have poor oxygen supply efficiency. These bioreactors were tested for production of biopesticides (Moser 1991) and dekalactone (Pakula and Freeman 1996); biological degradation of xenobiotics in wastewater (Radwan and Ramanujam 1997; Gavrilescu and Macoveanu 2000); and cultivation of animal cells (delos Santos *et al* 1994) and phototrophic organisms (Pirt *et al* 1983). In most cases the interior of tubular bioreactors was covered with microbial biomass in the form of a biofilm. The bioreactors with microbial biofilm have some advantages over the bioreactors with suspended cells – e.g. higher biomass concentrations (and consequently higher bioprocess productivity) and higher bioprocess stability to changes in the environment. The main disadvantage of the bioreactors with microbial biofilm is a relatively long period that is required for biofilm formation (Moser 1988).

The horizontal rotating tubular bioreactor (HRTB) combines the attributes of thin layer (Moser 1988) and biodisc reactor (Borchardt 1971; Venkataraman and Ramanujam 1998). The interior of HRTB was divided by O-ring shaped partition walls that served as carriers for microbial biomass. Mixing characteristics of HRTB were investigated previously in a broad range of combinations of process parameters: (i) bioreactor rotation speed; (ii) inflow (feeding) rate; (iii) liquid level in bioreactor; and (iv) distance between partition walls (Šantek *et al* 1996a,b, 1998a). The mixing efficiency was evaluated by the use of a temperature step method (Mayr *et al* 1992). For mixing description in HRTB, three mathematical models were established: 'simple' flow model (Šantek *et al* 1996a), 'spiral' flow model (Šantek *et al* 1996b, 1998a) and axial dispersion model (Šantek *et al* 2000). The comparison between these models showed that the "spiral" flow model was the most suitable for characterization of mixing in HRTB. To incorporate the 'spiral' flow model in semi-fundamental scale-up procedure, mathematical relations

Keywords. Acetate oxidation; bioprocess dynamics; horizontal rotating tubular bioreactor (HRTB); mixed microbial culture

between the adjustable model parameters and the bioreactor process parameters were developed. These mathematical equations were then used for the formation of prediction systems for adjustable model parameters and later were confirmed in the new experimental conditions (Šantek *et al* 1998b,c). To test performance of HRTB with a real biological system, an experimental model of fermentative glucose conversion with mixed microbial culture was established. In this experimental system we achieved very efficient glucose conversion into different products of metabolism (Ivančić *et al* 2004).

Main goal of this work was to examine the performance, as well as to find optimal conditions for conduction of aerobic microbial processes in HRTB. Acetate oxidation with mixed microbial culture was selected as a model system. During this investigation, microbial biofilm was formed on the inner surface of the bioreactor. Dynamics of the aerobic bioprocess in HRTB was examined for a broad range of combinations of process parameters: namely; bioreactor rotation speed, feeding rate, and acetate inlet concentration.

2. Materials and methods

2.1 *Microorganism, medium and growth conditions*

Microorganism was a mixed culture that was isolated from soil sample collected from Zagreb mountain. This microbial culture was adapted for acetate oxidation by repeated cultivation on rotary shaker, and by repeated batch cultivation in tank bioreactor. It was cultivated at room temperature ($21 \pm 1°C$) in liquid medium which contained (g l^{-1}): Na_2HPO_4 4·19; KH_2PO_4 1·50; NH_4Cl 2·00; $MgSO_4·7 H_2O$ 0·20; trace elements solution (ml l^{-1}) 4·00; and different acetate concentrations (1·4–7·2 g l^{-1}) added as a mixture of sodium acetate c_{NaAc} and acetic acid c_{HAc} to achieve pH in the range 6·2–6·5 (table 1). Trace element solution was prepared according to Robertson and Kuenen (1992). The solutions of CH_3COONa and CH_3COOH were sterilized by filtration separately, and the rest of the medium was sterilized by autoclaving (121°C for 25 min). These three medium components were mixed before use to achieve the required concentration.

Table 1. Concentration of sodium acetate (c_{NaAc}) and acetic acid (c_{HAc}) to achieve required acetate concentrations (c_{Ac}) and pH in cultivation medium.

c_{Ac} (g l^{-1})	1·4	2·9	4·3	5·8	7·2
c_{NaAc} (g l^{-1})	1·60	3·20	4·80	6·40	8·00
c_{HAc} (g l^{-1})	0·29	0·59	0·88	1·17	1·44
pH	6·49	6·40	6·38	6·37	6·28

2.2 *Bioreactor and experimental set-up*

HRTB was made of a plastic tube 1·8 m long with an inner diameter of 0·25 m. The interior of bioreactor was provided with a set of partition walls shaped as O-rings with outer diameter 0·25 m and inner diameter 0·19 m. Distance between the partition walls was 0·02 m. Liquid volume in HRTB was 10 l and liquid level 0·05 m, respectively. HRTB was horizontally placed on bearings that enabled rotation of the bioreactor (Šantek *et al* 1996a). Aeration was achieved through a perforated tube fixed in the central axis of HRTB. Air-flow rate was constant (172 l h^{-1}) throughout the experiment. Probes for sampling were placed through the length of the bioreactor, distanced every 0·45 m (figure 1). Required amount of suspended biomass for the inoculation of HRTB was obtained by repeated batch cultivation of the mixed culture in the tank bioreactor. This mixed culture was grown on the medium containing 7·2 g l^{-1} acetate. HRTB was inoculated with 9 l of culture from the tank reactor. Feeding rate of the fresh medium containing 7·2 g l^{-1} of acetate was adjusted on 1 l h^{-1}, and bioreactor rotation speed on 10 min^{-1}. To accelerate the biofilm formation, the culture was recirculated through the bioreactor for the first four days. Through that period, HRTB was re-inoculated from the tank reactor (that operated in repeated batch mode) three more times (6·5 l portions). It took 15 days, from the first inoculation, to achieve a stable biofilm formation in HRTB and then only it was considered as ready to conduct studies of its performance in different combinations of process conditions. These conditions were: (i) feeding rate 1–6·5 l h^{-1}, (ii) acetate inlet concentration 1·4–7·2 g l^{-1}, and (iii) bioreactor rotation speed 5–30 min^{-1}. In the first part of the experiment, effect of acetate inlet concentration and feeding rate on the acetate conversion efficiency were studied at constant bioreactor rotation speed ($n = 10$ min^{-1}). In the second part of the experiment, effect of the bioreactor process parameters (n and F) at constant inlet acetate concentration (2·9 g l^{-1}) was studied. Dynamics of the bioprocess in HRTB was monitored by withdrawing the samples from five positions through the length of the bioreactor, after five residence times from the establishment of new set of process parameters.

2.3 *Analytical methods*

Biomass concentration in suspension was determined by centrifugation of 10 ml sample for 20 min at 4500 rpm, washed twice with phosphate buffer (pH 8) and demineralized water and drying at 105°C for 48 h. The obtained supernatants were used for acetate and chemical oxygen demand (COD) determinations. The acetate concentration

was determined enzymatically by Boerhinger kits (Cat. No. 148 261), while COD by Merck kits (Cat. No. 1·4541·001 and 114555·0001), respectively. Mass of the biofilm was determined by collecting the biofilm sample from the inner bioreactor surface. The sample was suspended in phosphate buffer (pH 8), centrifuged and washed twice with phosphate buffer (pH 8) and demineralized water, and then dried at 105°C for 48 h. The acetate volumetric load was calculated as a ratio between acetate mass flow rate and working volume of HRTB. The acetate oxidation efficiency was calculated as a percentage of acetate that was used by mixed microbial culture in HRTB (concentration difference of inlet and outlet expressed as percentage of inlet).

3. Results and discussion

Acetate oxidation with mixed microbial culture was selected as a model system to study the performance of HRTB in a real aerobic bioprocess. For adequate air supply in HRTB, a perforated tube was incorporated along the bioreactor axis (figure 1). In preliminary experiments, effect of aeration on the acetate evaporation was examined. The medium with $7·2$ g l^{-1} acetate was aerated for 24 h (aeration rate 172 l h^{-1}). Reduction of the acetate concentration was observed to be only 2% of the initial concentration, suggesting that acetate loss due to evaporation ($0·006$ g l^{-1} h^{-1}) was insignificant compared to the acetate oxidation with microbial culture. HRTB was inoculated with mixed microbial culture that was propagated in a stirred tank reactor. During first 15 days part of microbial population gradually formed a stable biofilm on the interior surface of HRTB. During the period of the biofilm development the feeding rate was 1 l h^{-1}, the bioreactor rotation speed 10 min^{-1}, and acetate inlet concentration $7·2$ g l^{-1} respectively. After the biofilm had been developed, the studies on bioreactor performance started. The dynamics of process parameters changes during the whole experiment is presented in figure 2. In the first part of investigation (15–43 days), the effect of feeding rate and acetate inlet concentration on the acetate conversion efficiency was studied at constant bioreactor rotation speed ($n = 10$ min^{-1}). In the second part of the experiment (44–54 days) the effect of feeding rate and bioreactor rotation speed on the bioprocess efficiency was examined at constant acetate inflow concentration ($2·9$ g l^{-1}).

In previous research it was proved that bioreactor rotation speed higher than 30 min^{-1} produces intensive biofilm detachment (Ivančić *et al* 2004). During this research it was also observed that feeding rate higher than $6·5$ l h^{-1} produced considerable biomass washout due to biofilm erosion. Therefore each of these two values were chosen as maximum of the investigated range of process para-
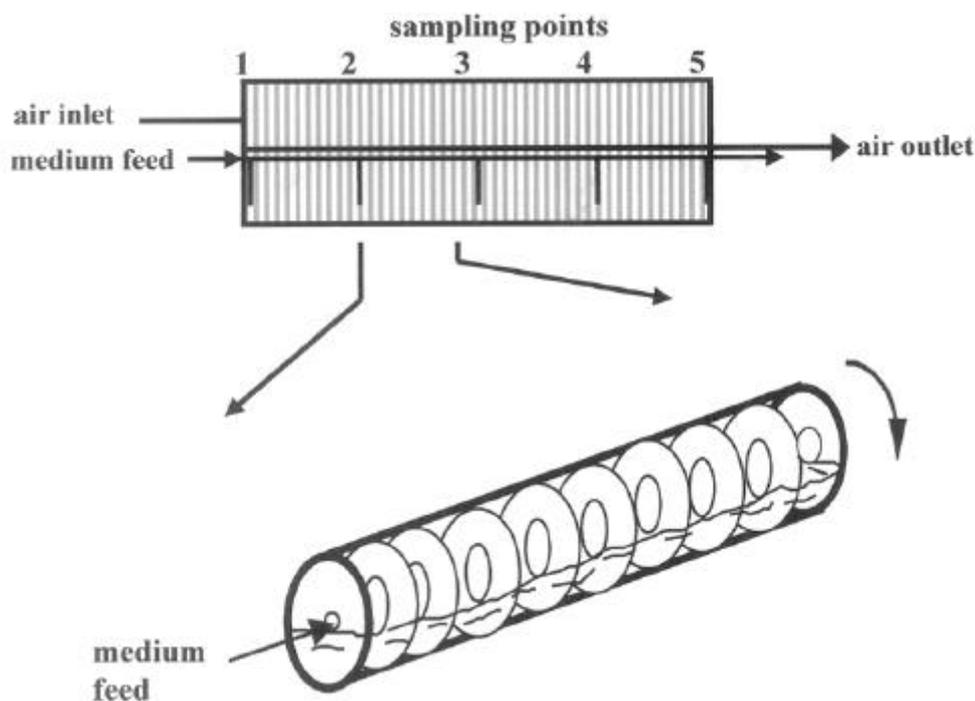

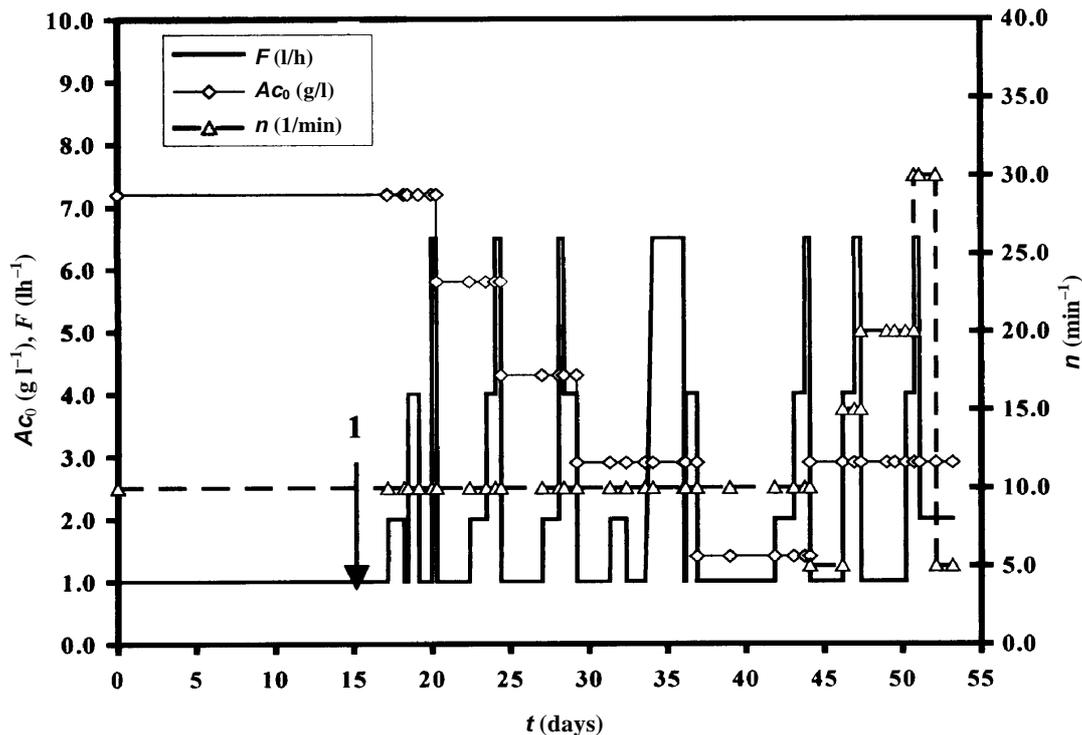**Figure 1.** A diagram of horizontal rotating tubular bioreactor.

Figure 2. Dynamics of process parameter changes [acetate inlet concentration (Ac_0), feeding rate (F) and bioreactor rotation speed (n)] during the whole experiment in HRTB. Arrow 1 represents the end of biofilm formation and beginning of bioprocess investigation.

meters. In the first part of experiment conducted at constant bioreactor rotation speed, the acetate concentration was monitored along HRTB at different feeding rates ($1–6.5 \, l \, h^{-1}$), and acetate inlet concentrations ($1.4–7.2 \, g \, l^{-1}$). Two examples of acetate concentration profiles along HRTB are presented in figures 3 and 4 (acetate concentration $1.4 \, g \, l^{-1}$ and $7.2 \, g \, l^{-1}$, respectively). As it can be seen in figure 3 the acetate concentration in the first measuring point of HRTB (0% of L_{HRTB}) varies in great extent due to dilution of feeding medium with HRTB liquid content and consumption of acetate by the biomass in HRTB. These variations were highly influenced by the feeding rate: higher the feeding rate – higher the acetate concentration at first measuring point in HRTB (more close to the acetate concentration in the feed). In this set of experiments (acetate concentration in the feed $1.4 \, g \, l^{-1}$), complete acetate consumption was observed for feeding rates up to $4 \, l \, h^{-1}$. Moreover, at these feedings rates a complete acetate consumption was achieved at less than 75% of HRTB length. Increasing of feeding rate to $6.5 \, l \, h^{-1}$ led to significant reduction of acetate consumption. For acetate inlet concentration of $7.2 \, g \, l^{-1}$ (figure 4), a very small decrease of acetate concentrations was detected along HRTB. This effect can be explained by the fact that many microorganisms are sensitive to relatively low acetate concentrations ($1.0 \, g \, l^{-1}$) (Fukaya *et al* 1992).
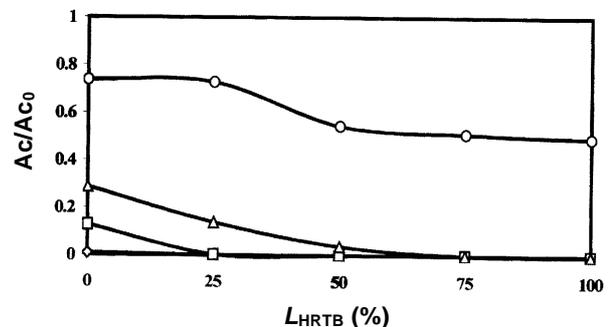

Figure 3. Changes of Ac/Ac_0 ratio along HRTB at different feeding rates [$F = 1.0 \, l \, h^{-1}$ (◊), $F = 2.0 \, l \, h^{-1}$ (□), $F = 4.0 \, l \, h^{-1}$ (△), $F = 6.5 \, l \, h^{-1}$ (○)] and constant acetate inlet concentration ($Ac_0 = 1.4 \, g \, l^{-1}$).

Liquid flow in tubular bioreactors is characterized by plug flow conditions. Consequently, gradients of concentrations, and/or temperature are formed along the bioreactor length (Moser 1985, 1988). During hydrodynamic investigations in the HRTB, it was observed that liquid flow in the bioreactor was characterized by plug flow conditions (Šantek *et al* 2000). The observation of acetate gradients along HRTB length confirmed the assumption of the plug flow conditions. As a consequence of acetate

degradation, pH changes along the HRTB length were observed as well (figures 5 and 6; same experimental conditions as figures 3 and 4 respectively). At higher feeding rates, lower pH values were observed as a consequence of higher acetate loads. Significant acetate consumption (figure 3) and small pH change (figure 5) along HRTB length were observed at acetate inlet concentration of $1 \cdot 40$ g l^{-1}. The high acetate consumption, the mixing intensity in the bioreactor and buffering capacity of the medium were the main reasons for previously mentioned effect. In case of acetate inlet concentration of $7 \cdot 20$ g l^{-1} and lower feeding rates (1 and $2 \, l \, h^{-1}$) an opposite effect was observed: i.e. relatively small acetate consumption (figure 4) was related to the considerable increase of pH profiles (figure 6) along HRTB length. This effect can be explained by biofilm detachment observed during this particular measurement.

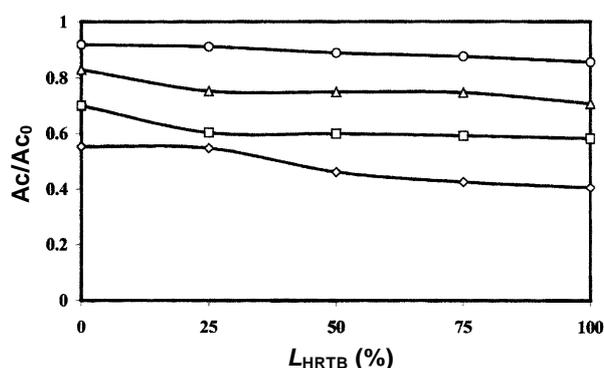The dependence of the overall efficiency of acetate oxidation on feeding rate and acetate inlet concentration is presented in figure 7 for the entire range of investigated process parameters. Obtained results indicate that critical parameter for HRTB performance is volumetric load of acetate (product of feeding rate and inlet concentration divided by liquid volume in HRTB). The result of figure 7 is presented in figure 8 as a function of acetate volumetric load. The efficiency of acetate oxidation was in the range of 80–100% for acetate volumetric load of $0 \cdot 14$–$0 \cdot 58$ g acetate l^{-1} h^{-1}. These volumetric loads correspond to the lower acetate inlet concentrations ($1 \cdot 4$–$2 \cdot 9$ g l^{-1}) and feeding rates (1–$2 \, l \, h^{-1}$). Further, increase of volumetric load ($0 \cdot 6$–$1 \cdot 7$ g acetate l^{-1} h^{-1}) provoked the decrease of the efficiency of acetate oxidation to the level of 50–75%. At acetate inlet concentration in the range of $5 \cdot 8$–$7 \cdot 2$ g l^{-1} and inflow rates 4–$6 \cdot 5 \, l \, h^{-1}$, the efficiency of acetate oxidation was in the range of 17–50%. In these conditions acetate volumetric load was higher than 2 g acetate l^{-1} h^{-1}. During this investigation (data not shown) it was also observed that increase of acetate volumetric load up to 2 g l^{-1} h^{-1} was related to the approximately linear increase of acetate consumption rate. At volumetric loads higher than 2 g l^{-1} h^{-1}, microbial biofilm stability was disturbed due to effects of acetate inhibition. COD determinations in outlet samples were almost identical to results of acetate analysis (correlation coefficient $0 \cdot 9977$, data not shown). According to the fact that theoretically 1 g l^{-1} of acetate corresponds to $1 \cdot 0667$ g l^{-1} COD, one can conclude that acetate was the single organic substance in the samples, indicating that no metabolites were formed during experiment and that complete oxidation of acetate into carbon dioxide and water occurred. Biofilm age and environmental conditions are also significant factors that have great impact on the activity and stability of microbial biofilm (Moser 1988). Microbial biomass grows in a form of suspended single cells, suspended cell clusters, and a biofilm attached to the bioreactor inner surface. During this research suspended biomass dry weight along HRTB was

Figure 4. Changes of Ac/Ac_0 ratio along HRTB at different feeding rates [$F = 1 \cdot 0 \, l \, h^{-1}$ (◊), $F = 2 \cdot 0 \, l \, h^{-1}$ (□), $F = 4 \cdot 0 \, l \, h^{-1}$ (△), $F = 6 \cdot 5 \, l \, h^{-1}$ (○)] and constant acetate inlet concentration ($Ac_0 = 7 \cdot 2$ g l^{-1}).
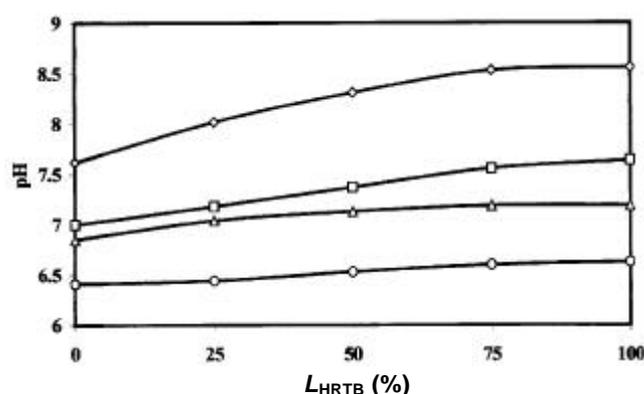

Figure 5. Changes of pH along HRTB at different feeding rates [$F = 1 \cdot 0 \, l \, h^{-1}$ (◊), $F = 2 \cdot 0 \, l \, h^{-1}$ (□), $F = 4 \cdot 0 \, l \, h^{-1}$ (△), $F = 6 \cdot 5 \, l \, h^{-1}$ (○)] and constant acetate inlet concentration ($Ac_0 = 1 \cdot 4$ g l^{-1}).

Figure 6. Changes of pH along HRTB at different feeding rates [$F = 1 \cdot 0 \, l \, h^{-1}$ (◊), $F = 2 \cdot 0 \, l \, h^{-1}$ (□), $F = 4 \cdot 0 \, l \, h^{-1}$ (△), $F = 6 \cdot 5 \, l \, h^{-1}$ (○)] and constant acetate inlet concentration ($Ac_0 = 7 \cdot 2$ g l^{-1}).
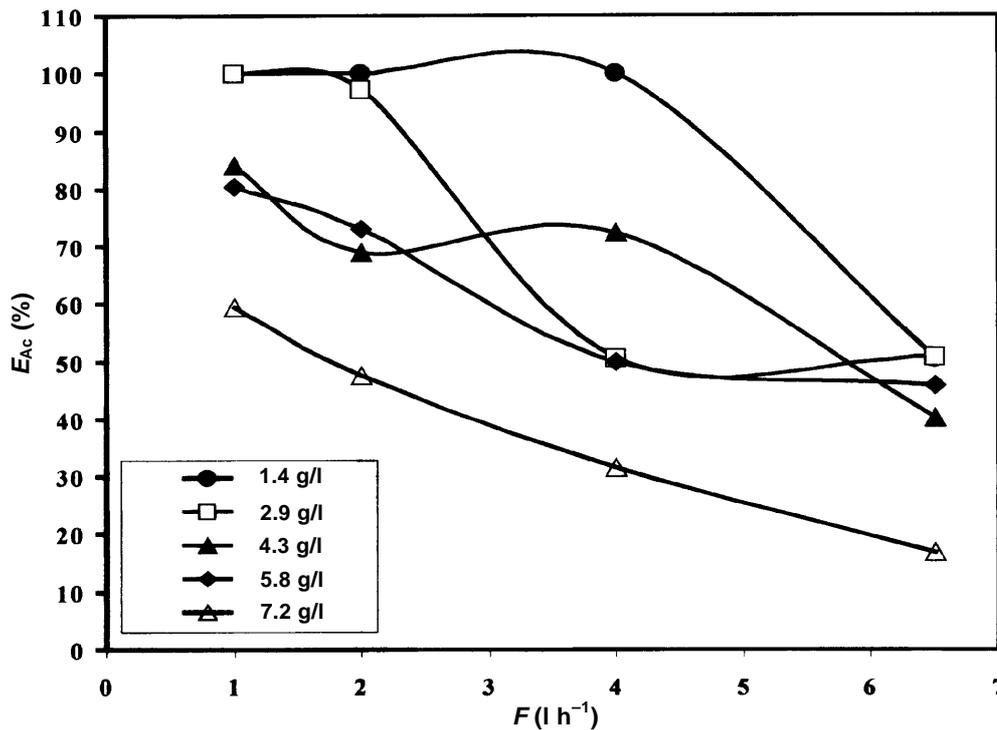
Figure 7. Relation between the efficiency of acetate oxidation (E_{Ac}) and feeding rate (F) at different acetate inlet concentrations (Ac_0).

monitored and depending on the experimental conditions it was in the range of 4·0–9·8 g l^{-1}. Obtained profiles of suspended biomass along HRTB were mostly at approximately constant level. In a few cases, observed changes of suspended biomass along HRTB length were stochastically most probably a consequence of more intensive biofilm detachment (erosion and sloughing) process (Stewart 1993; Peyton and Characklis 1993). Biofilm detachment is a complex process affected by hydrodynamic conditions together with morphological and physiological character- istics of the biofilm (Gavrilescu and Macoveanu 2000; Gavrilescu 2002). During this investigation, the thickness of the formed microbial biofilm was approximately con- stant (1·0 mm) except in few cases when more intensive detachment was observed, particularly at highest feeding rate (6·5 l h^{-1}). The average dry weight of the biofilm on the inner surface of HRTB was 14·90 g m^{-2}. This could be considered as low biofilm density compared to the literature data (Venkataraman and Ramanujam 1998). In the second part of the experiment (44–54 days), the effect of bioreactor rotation speed and feeding rate on the efficiency of acetate consumption was studied at constant acetate inlet concentration (2·9 g l^{-1}). Obtained efficiency of acetate oxidation for different combinations of process parameters (n and F) is presented in table 2. Complete

acetate oxidation was observed for lower feeding rates ($F = 1$–2 l h^{-1}) by all bioreactor rotation speeds ($n = 5$– 30 min^{-1}). The increase of feeding rate at 4–6·5 l h^{-1} was connected with significant decrease of the efficiency of acetate oxidation (45·5–65·5%). At these feeding rates, more pronounced effect of bioreactor rotation speed on the efficiency of acetate oxidation was detected. In these conditions, further increase of bioreactor rotation speed ($n > 10$ min^{-1}) was related to the increase of bioprocess efficiency. This effect is most probably a consequence of higher mixing intensity. The effect of bioprocess para- meters (n and F) on normalized acetate concentrations at front position in HRTB (0% L_{HRTB}) is presented in figure 9 and at end position (100% L_{HRTB}) in figure 10, respec- tively. The increase of feeding rate is related to the increase of normalized acetate concentration as a conse- quence of higher acetate loads in these conditions. The bioreactor rotation speed had also an important effect on the efficiency of acetate oxidation (most obvious at higher bioreactor rotation speed). Obtained results clearly showed that along HRTB length, efficient acetate oxida- tion took place for different combinations of process parameters. Our results are in agreement with results of hydrodynamic investigations in the HRTB by Šantek *et al* (1996a,b 1998a).
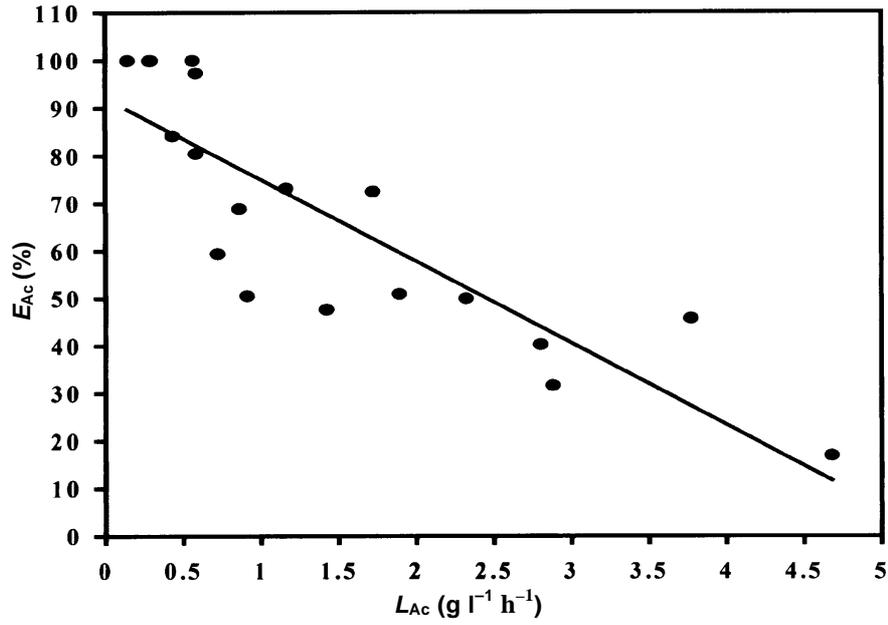
Figure 8. Relation between the efficiency of acetate oxidation (E_{Ac}) and acetate volumetric load (L_{Ac}). Straight line represents linear approximation of experimental data.
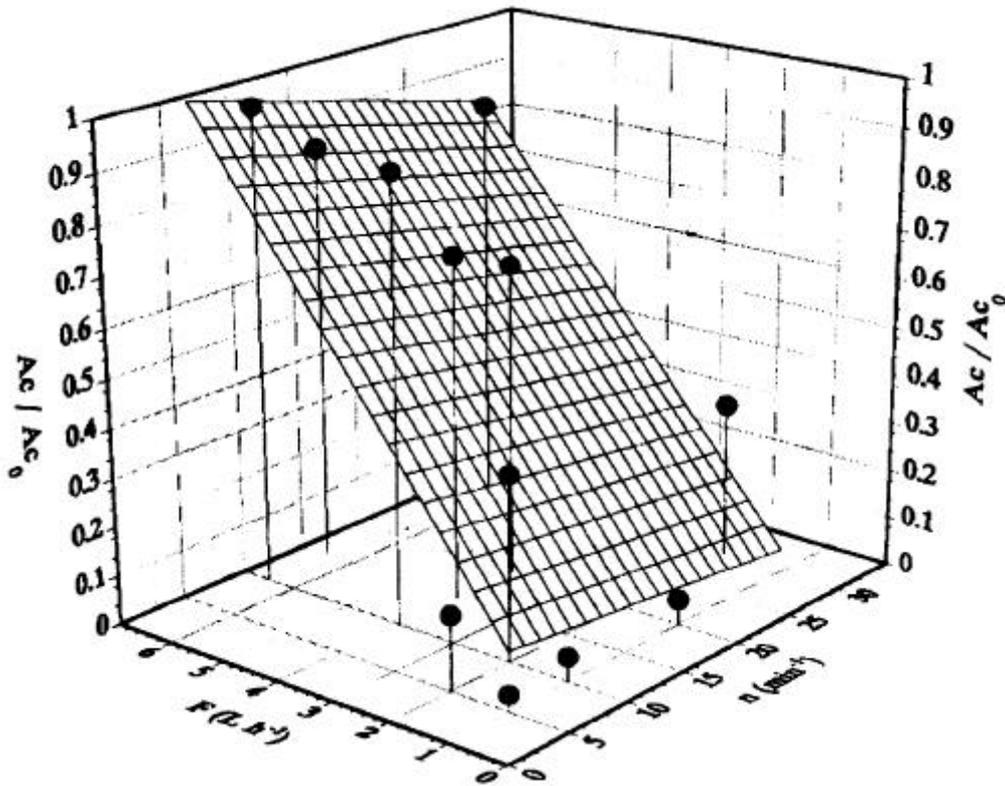

Figure 9. Ac/Ac_0 ratio as a function of the feeding rate (F) and bioreactor rotation speed (n) at front position in HRTB (0% L_{HRTB}). Points represent experimental data and surface is mathematical approximation of these data. (Acetate inlet concentration $2 \cdot 9$ g l^{-1}).

Figure 10. Ac/Ac_0 ratio as a function of the feeding rate (F) and bioreactor rotation speed (n) at end position in HRTB (100% L_{HRTB}). Points represent experimental data and surface is mathematical approximation of these data. (Acetate inlet concentration 2·9 g l^{-1}).

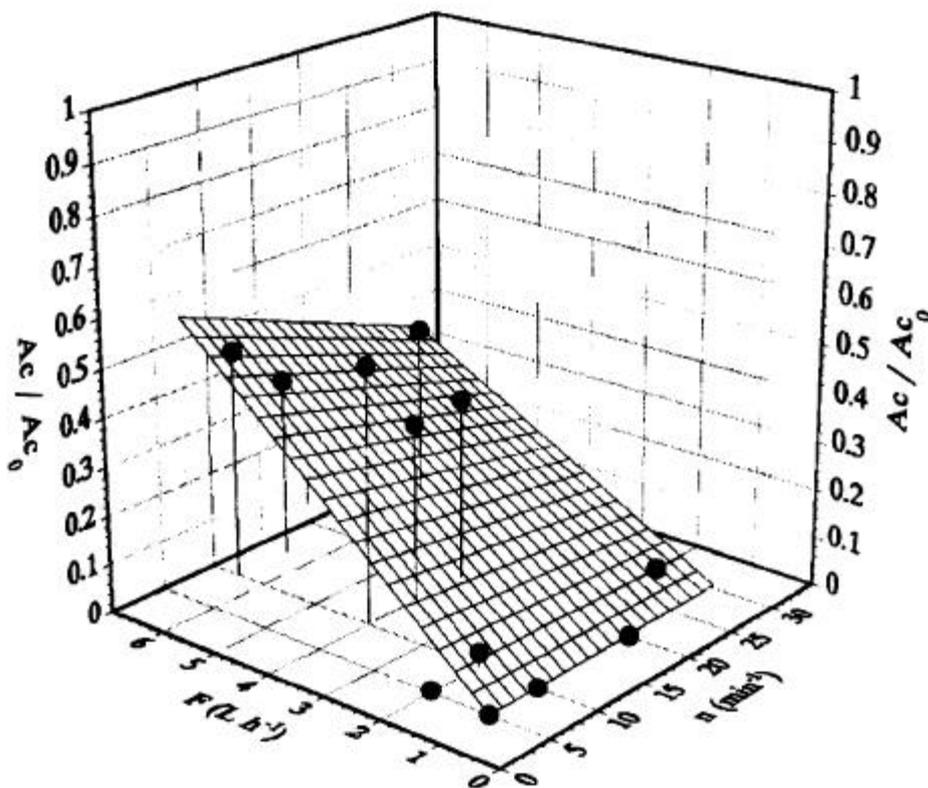**Table 2.** Efficiency of acetate oxidation [E_{Ac} (%)] by different combinations of process parameters (n and F) and constant acetate inlet concentration ($Ac_0 = 2.9$ g l^{-1}).

F (l h^{-1})	n (min^{-1})				
	5	10	15	20	30
1·0	100	100	–	100	–
2·0	96·55	97·28	–	–	100
4·0	–	45·48	64·76	63·72	–
6·5	–	50·86	64·24	–	65·62

4. Conclusions

In this work, the capability of HRTB to conduct aerobic processes was tested on a model system of aerobic acetate oxidation by the action of a mixed microbial culture. The microbial culture grew in suspension and as a biofilm on the interior surface of HRTB. The profiles of suspended biomass concentration along HRTB length and biomass in biofilm were at approximately constant level during the experiment. The obtained results suggest that acetate inlet concentration and feeding rate [and the product of two (the acetate volumetric load)] have significant effect on the efficiency of acetate oxidation. The effect of bioreactor rotation speed on the bioprocess dynamics was less pronounced, but still observable at higher rotation speed. Acetate concentration and pH gradients along HRTB were more expressed at lower feeding rates. At volumetric loads above 2 g l^{-1} h^{-1}, acetate oxidation efficiency was only 17 to 50%. When volumetric load was in the range of 0·60–1·75 g acetate l^{-1} h^{-1}, acetate oxidation efficiency was 50–75%. At lower volumetric loads (0·14–0·58 g acetate l^{-1} h^{-1}), total acetate consumption was observed. Thus, obtained results clearly show that HRTB is suitable for conducting the aerobic bioprocesses.

References

Borchardt J A 1971 Biological waste treatment using rotating discs; *Biotechnol. Bioeng. Symp.* **2** 131–140

delos Santos B, Honda H, Shiragami N, Kariya M and Unno H 1994 Simulated-microcarrier motion and its effect on radial medium transfer inside horizontally rotating cylindrical bioreactor (HRCB) for animal cell culture; *Bioproc. Eng.* **10** 5–14

Fukaya M, Park Y S and Toda K 1992 Improvement of acetic acid fermentation by molecular breeding and process development; *J. Appl. Bacteriol.* **73** 447–454

Gavrilescu M 2002 Engineering concerns and new developments in anaerobic wastewater treatment; *Clean Techn. Environ. Policy* **3** 346–362

Gavrilescu M and Macoveanu M 2000 Attached-growth process engineering in wastewater treatment; *Bioproc. Eng.* **23** 95–106

Ivančić M, Šantek B, Novak S and Marić V 2004 Fermentative bioconversion in a horizontal rotating tubular bioreactor; *Proc. Biochem.* **39** 995–1000 (URL: http://www.elsevier.com/locate/procbio)

Mayr B, Horvat P and Moser A 1992 Engineering approach to mixing quantification in bioreactors. *Bioproc. Eng.* **8** 137–143

Moser A 1985 Imperfectly mixed bioreactor systems; in *Comprehensive biotechnology* (ed.) M Moo-Young (Oxford: Pergamon Press), vol. 2, pp 77–98

Moser A 1988 *Bioprocess technology – Kinetics and reactors* (New York-Wien: Springer)

Moser A 1991 Tubular bioreactor: case study of bioreactor performance for industrial production and scientific research; *Biotechnol. Bioeng.* **37** 1054–1065

Pakula R and Freeman A 1996 A new continuous biofilm bioreactor for immobilised oil-degrading filamentous fungi; *Biotechnol. Bioeng.* **49** 20–25

Peyton B M and Characklis W G 1993 A statistical analysis of the effect of substrate utilisation and shear stress on the kinetics of biofilm detachment; *Biotechnol. Bioeng.* **41** 728–735

Pirt J S, Lee Y K, Walach M K, Pirt M W, Balyuzi H H M and Bazin M J 1983 A tubular bioreactor for photosynthetic production of biomass from carbon dioxide: design and performance; *J. Chem. Tech. Biotechnol.* **33** 35–39

Radwan K H and Ramanujam T K 1997 Studies on organic removal of 2,4-dichlorophenol in wastewaters using a modified RBC; *Bioproc. Eng.* **16** 219–223

Robertson L A and Kuenen J G 1992 The effect of electron acceptor variations on the behaviour of *Thiosphaera pantotropha* and *Paracoccus denitrificans* in pure and mixed culture; *FEMS Microbiol. Ecol.* **86** 221–228

Šantek B, Horvat P, Novak S, Mayr B, Moser A and Marić V 1996a Mathematical model of mixing in a horizontal rotating tubular bioreactor: "Simple flow" model; *Bioproc. Eng.* **14** 195–204

Šantek B, Horvat P, Novak S, Mayr B, Moser A and Marić V 1996b Mathematical model of mixing in a horizontal rotating tubular bioreactor: "Spiral flow" model; *Bioproc. Eng.* **14** 223–229

Šantek B, Horvat P, Novak S, Moser A and Marić V 1998a Studies on mixing in horizontal rotating tubular bioreactor: I. Optimisation of adjustable parameters for "spiral flow" model; *Bioproc. Eng.* **18** 467–473

Šantek B, Horvat P, Novak S, Moser A and Marić V 1998b Studies on mixing in horizontal rotating tubular bioreactor: II. Prediction systems for adjustable parameters of "spiral flow" model; *Bioproc. Eng.* **19** 19–28

Šantek B, Horvat P, Novak S, Moser A and Marić V 1998c Studies on mixing in horizontal rotating tubular bioreactor: III. Influence of liquid level and distance between the partition walls on prediction systems for adjustable model parameters; *Bioproc. Eng.* **19** 91–102

Šantek B, Horvat P, Novak S, Sunko D, Moser A and Marić V 2000 Estimation of axial dispersion in horizontal rotating tubular bioreactor by means of a structured model; *Bioproc. Eng.* **23** 265–274

Stewart P S 1993 A model of biofilm detachment; *Biotechnol. Bioeng.* **41** 111–117

Venkataraman R and Ramanujam T K 1998 A study of microbiology of biological film layer in rotating biological contactors; *Bioproc. Eng.* **18** 181–186