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Performance of *Aspergillus niger* Cultivation in Geometrically Dissimilar Bioreactors Evaluated on the Basis of Morphological Analyses

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Summary

The growth of *Aspergillus niger*, citric acid production and mycelia morphology changes were compared under different mixing conditions in bioreactors with two types of stirrers: Rushton turbine stirrers (RTS1 or RTS2) and axial counterflow stirrers (ACS1 or ACS2).

The characteristics of growth, productivity and morphology varied with the mixing system and the applied agitation regime. In the first series of experiments, the flow characteristics of *Aspergillus niger* broth under different mixing conditions were analysed in a model bioreactor using RTS1 and ACS1. The kinetic energy E of flow fluctuations was measured in gassed and ungassed water and fermentation broth systems using a stirring intensity measuring device (SIMD-f1). The difference of energy E values at different points was more pronounced in the bioreactor with RTS1 than in the case of ACS1. High viscous *A. niger* broths provided higher energy E values in comparison with water. It was observed that the *Aspergillus niger* growth rate and citric acid synthesis rate decreased at very high energy E values, the behaviour obviously being connected with the influence of the irreversible shear stress on the mycelial morphology.

In the second series of experiments, a higher citric acid yield was achieved in the case of ACS2 at a power input approximately twice lower than in the case of RTS2. Morphological characterization of *A. niger* pellets was carried out by the image analysis method. ACS2 provided the development of morphology, where pellets and cores had larger area, perimeter and diameter, and the annular region of pellets was looser and more »hairy« in comparison with the case of RTS2. The pellets from the fermentation with RTS2 were smaller, denser, with shorter hyphae in the annular region of pellets, and the broth was characterized by a higher percentage of diffuse mycelia.

Power input studies of RTS2 and ACS2 were made at different agitator rotation speeds and gas flow rates using water and *Aspergillus niger* broths. RTS2 was a high power number mixing system ($P_O = 6.8$), whose P_O was practically invariable in different media under study. For ACS2, P_O increased approximately 3 times from 0.68 in water to 2.11 in high viscous *Aspergillus niger* broth. The effect of the agitation and aeration rates on the power input of RTS2 and ACS2 was analysed. ACS2 proved to be more effective, since it lost much less power due to aeration.

Key words: geometrically dissimilar bioreactors, *Aspergillus niger*, citric acid, morphology, stirring intensity measuring device, flow energy distribution

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Introduction

Problems of mass exchange in the liquid-cell-liquid system have been studied for decades. There is enormous literature on the optimization of aeration and mixing modes, the internal design of bioreactors, *etc.*, that accounts for hundreds of references annually *(1–4)*, including our own publications *(5–12)*. The results of our studies in this area show that intense modes of aeration and mixing inevitably lead to turbohypobiosis. The deformation damage of cells in intensively mixed zones proved to be significantly more dangerous than the insufficient mass exchange in the so-called dead zones of bioreactors. In our experiments, these situations were simulated by two fundamentally different systems of stirring, *i.e.* the standard radial blade – Rushton turbine stirrer (RTS) and the axial counterflow stirrer (ACS).

A special bioreactor design was performed to provide mycelial and other producers sensitive to deformation forces with even mixed cultivation conditions *(13)*. Axial mixing systems, *e.g.* a counterflow system, were shown to be most promising in this context *(8,9,14)*.

We also tried to find simple and instrumentally measurable criteria for cultivation scaling in these geometrically dissimilar systems. A special stirring intensity measuring device (SIMD-f1) was developed for this purpose, and a number of mycelial cultures with high viscosity and non-Newtonian hydrodynamic parameters were tested as biological models *(6,8,9,11,14–20)*. However, we failed to find such criteria, even if the morphological structure of the population was measured by an automatic analyser, and fluctuations of energy *E* were monitored with a special probe. We had to agree with the known conclusion that each specific system (culture + bioreactor) requires individual adjustment and optimization of the cultivation regime.

However, there is strong evidence that the mixing and/or aeration intensity and limiting of concentration of substrate contribute alternatively (within reasonable ranges of variation) to the efficiency of bioreactors supplied with a gas mixture (air, nitrogen and oxygen).

The present work comprises the results of the optimization and adjustment of the mycelial systems mentioned above, based on morphological analyses.

Mycelial cultures, which usually refer to morphologically complex microorganisms, are particularly sensitive to hydrodynamic conditions and have various reactions to mixing and flow distribution conditions. Different morphological growth forms (pelleted or freely dispersed ones) can have a significant effect on the rheology of the fermentation broth and thus the performance of the bioreactor. *A. niger* usually grows in the form of mycelial agglomerates, nevertheless, filamentous growth is also found to be highly productive *(21,22)*. At too high agitator rotation speeds, mycelial agglomerates are frequently disintegrated, and high viscous filamentous mycelia are formed, causing oxygen transfer problems as well as difficulties of biomass separation from the fermentation broth *(8,9)*.

Traditional methods of hydrodynamic analysis do not always lead to the valid results for mixing optimization. Therefore, it is necessary to look for new approaches *(23)*. The use of a stirring intensity measuring device, SIMD-f1, *(11,17,20)* enables measurements of the local flow kinetic energy *E* in multiphase media and could be one of the solutions in the analysis of the flow character in a bioreactor.

The aim of the present investigation was to study the effect of geometrically dissimilar bioreactors on *A. niger* fermentation performance and culture morphology, as well as to correlate the hydrodynamic characteristics of flows measured by SIMD-f1 in bioreactors during the cultivation.

Materials and Methods

Microorganism and culture conditions

The citric acid producing fungus *Aspergillus niger R3* strain from the collection of the Experimental Plant of Biochemical Preparations, Latvia, was used. The inocula preparation and media composition were as described previously *(5,8)*. The initial spore concentration of the inoculum was approximately $3.5 \cdot 10^6$ spores/mL. The inoculum was grown in shake flasks in a shaker incubator (220 rpm) for 24 h at 32 °C using the medium containing: 100 g/L glucose, 2.5 g/L NH_4NO_3, 0.16 g/L KH_2PO_4, 0.25 g/L $MgSO_4 \cdot 7H_2O$, 8 g/L molasses, 5 mL of trace element solution, up to 1 L of distilled water, pH=5.0. Fermenter cultures were inoculated with 10 % (v/v) of the mycelium suspension from the shake flasks. The fermentation medium contained: 120 g/L glucose, 0.6 g/L NH_4NO_3, 1.0 g/L urea, 0.16 g/L KH_2PO_4, 0.5 g/L $MgSO_4 \cdot 7H_2O$, 0.075 g/L KCl, 0.01 g/L $ZnSO_4 \cdot 7H_2O$, 0.02 g/L $MnSO_4 \cdot 4H_2O$, 5 mL/L of trace element solution, up to 1 L of distilled water, pH=5.6. The trace element solution contained: 20 mg KI, 17.25 mg $Na_2B_4O_7 \cdot 10H_2O$, 6.8 mg $MnCl_2 \cdot 4H_2O$, 7.8 mg $CuSO_4 \cdot 5H_2O$, 3.55 mg $(NH_4)_6Mo_7O_{24} \cdot 4H_2O$, 66.8 mg $(NH_4)_2SO_4 \cdot FeSO_4 \cdot 6H_2O$, distilled water up to 1 L, pH=2.5.

The medium temperature in bioreactors was controlled at (32.0 ± 0.3) °C. The duration of fermentations was 7 days.

In the first series of experiments, flow characteristics of *A. niger* fermentation broths and water, when applying different mixing conditions, were analysed using the stirring intensity measuring device, SIMD-f1 *(11,16–18,20)*. SIMD consists of piezoelectric transducer and data processing unit, and its working principle is based on the transformation of the mechanical interaction of the flow and probe into electrical signals, which are processed by the data processing unit. The latter ensures the measuring and monitoring of the kinetic energy *E* of flow fluctuations in specific energy units (J/m^3).

Flow distribution measurements were made in a model bioreactor with a working volume of 3 L (*24*). The diameter and height of the model bioreactor were 140 and 260 mm, respectively. Flow distribution measurements in a model bioreactor were made by two geometrically dissimilar double-tier stirring systems, RTS1 and ACS1. The height and diameter of the RTS1 stirrer were 20 and 73 mm, respectively. The height and diameter of the ACS1 stirrer were 16 and 75 mm, respectively. In ACS1, two impellers were mounted on the

drive axis. During the impeller rotation, the axial flows from both impellers were in mutually opposite directions. This was achieved using impellers consisting of 6 wing-shaped blades and a diffuser (height = 15 mm). The blades of the upper and lower impellers were bent at equal angles (α = 25°) but in correspondingly opposite directions from the horizontal plane of reference. The distance between the central planes of the impellers, and the distance between the middle plane of the lower impeller and the base of the bioreactor were equal to the diameter of each mixing system. The model bioreactor had three evenly spaced baffles (width = 20 mm).

The second series of *A. niger* fermentations, where morphological measurements were made by image analysis, was carried out in 6 L bioreactors with a working volume of 5 L (Life Science Laboratories Ltd., Luton, Great Britain) with a Rushton turbine stirrer (RTS2) and an axial counterflow stirrer (ACS2). The RTS2 consisted of 6 blades, and the total impeller diameter was 76.5 mm. The ACS2 consisted of impellers with 4 wing--shaped blades. The blades of the upper and lower impellers of the pair were bent at equal angles (α = 28°) but in correspondingly opposite directions from the horizontal plane of reference. The diameter of ACS2 was 87.5 mm. The agitator rotation speeds during the citric acid production phase for RTS2 and ACS2 were 500 rpm (tip speed 2.0 m/s) and 400 rpm (tip speed 1.8 m/s), respectively. The air flow rates during the citric acid production phase were 0.5 vvm.

The biomass and citric acid concentrations in the first run were analysed as described by Vanags *et al. (8)*, and in the second run, as described by Bujalski *et al. (5)*.

The rheological characteristics of *A. niger* fermentation broths in the second run were determined using the Rheomat 30 *(5)*.

Image analysis

Samples of the *A. niger* fermentation broth were stained with lactophenol cotton blue. Then the sample was diluted with water and placed into a Petri dish. A macroviewer Kaiser RT1-5450 (Germany) connected to a Video CCD camera SANYO (Sanyo, Japan) was used for image analysis of *A. niger* pellets.

The morphological measurements of *A. niger* pellets were carried out using the method and software developed by Cox and Thomas *(25,26)*. The mean morphological characteristics of mycelial pellets and cores were: area (the area of hyphae in the pellet or core), convex area (the area after the elimination of concavities in the pellet or core perimeter), and equivalent pellet or core diameter (the diameter of a circle of the same area as the whole pellet or core).

Results

The analysis of *A. niger* fermentation performance using different stirring systems showed that high citric acid concentrations were achieved with stirring systems providing good mass transfer conditions without the damage of mycelia *(5,8,9)*. The axial counterflow stirrer was found to be more effective than the Rushton turbine stirrer, hence, a higher citric acid concentration was achieved with ACS1 to be 82 g/L, while that in the case of RTS1 was 75 g/L *(8)*. A higher agitator rotation speed of the axial counterflow stirrer (750 rpm) caused less damage of mycelia in comparison with the case of the turbine stirrer (600 rpm). In experiments where Rushton turbine stirrer was used, oblong, spindle-type pellets were formed, their number being increased from approximately 1500 pellet/mL on the third day to 2000–2500 pellet/mL at the end of fermentation. Some proportion of freely dispersed mycelia was formed starting with the fifth day, decreasing the citric acid synthesis rate. When an axial counterflow stirrer was used, only small oblong pellets (1000–1500 pellet/mL) were formed in the broth without the formation of a diffuse mycelium.

In order to compare flow characteristics in bioreactors with turbine or axial counterflow stirrers, flow energy distribution at different stirrer rotation speeds and air flow rates were analysed for water and *A. niger* fermentation broths using SIMD-f1. The energy *E* distribution was analysed in the planes of maximal mixing in-

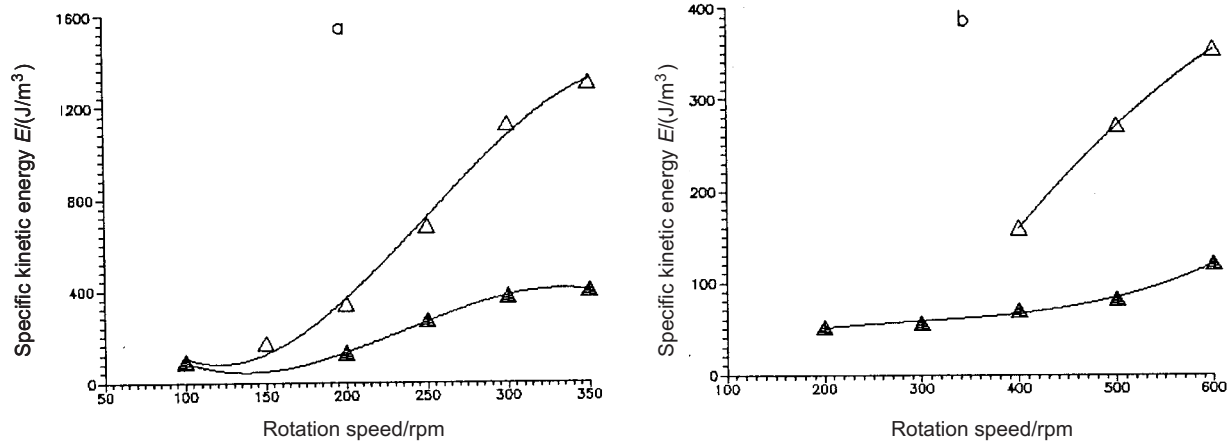

Fig. 1. Effect of agitator rotation speed on the distribution of energy *E* in a definite point of the fermerter with RTS1 (a) and ACS1 (b) in gassed (1 vvm) water (▲—▲) and fermentation broth of *A. niger* (Δ—Δ). The reference line of the height *z* and the distance from the centre *r*: *z* = 0 mm and *r* = 41.2 mm, respectively, for RTS1; *z* = 12.5 mm and *r* = 37.5 mm, respectively, for ACS1

tensity. In both stirring systems, the zones of maximal mixing intensity were determined experimentally by measuring energy E at different points of the bioreactors. For RTS1, the zone of maximal flow intensity was found to be in the middle plane z of the upper impeller opposite the end of the blades (r = 41.2 mm, z = 0 mm). For ACS1, this zone was just above the tip of the blades (5 mm above the diffuser) of the lower impeller (r = 37.5 mm, z = 12.5 mm). In these points, energy E changes were analysed for water and fermentation broths of *A. niger*. As can be seen from Fig. 1, energy E values increased with the increase of the agitator rotation speed for both the mixing systems and were higher for *A. niger* broths in comparison with water, demonstrating different liquid and gas flow character and gas dispersion.

Figs. 2 and 3 show substantial differences in the distribution of energy E for water or *A. niger* broth when different stirring systems were used. Higher energy E values for high viscosity *A. niger* broth in comparison with water was an overall tendency in the local intensive zones. It could be due to a higher viscosity of *A. niger* broth. The elevated energy E values in the local intensive planes of RTS1 using *A. niger* broth could indicate an increased shear stress in this zone, which obviously caused undesirable morphological changes in the culture. More rapid decrease of energy E occurred with the increase of the distance from the impeller to the wall of the bioreactor due to a high culture viscosity. In fact, a very viscous medium showed a very intensive mixing around the impeller, while near the wall of the bioreactor, the broth was practically stagnant.

Differences in energy E values at different air flow rates were particularly pronounced in the case of RTS1 using water. In the case of ACS1, a tendency of higher energy E values for aerated *A. niger* fermentation broth in comparison with unaerated medium was observed. The interaction of two opposite effects was observed: the decrease of energy E with the increase of aeration (connected with the decrease in the introduced power) and the increase in the intensity of the axial flow above

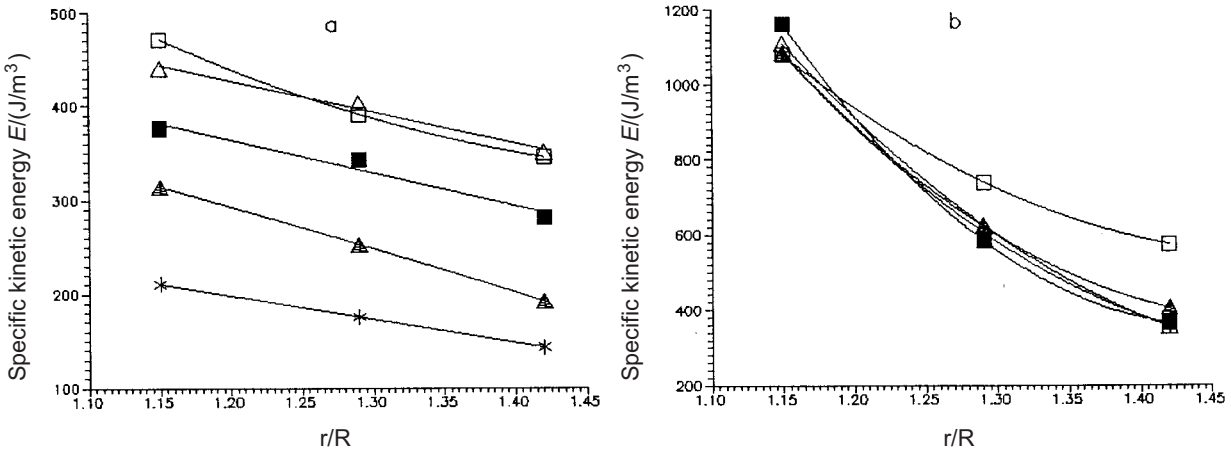

Fig. 2. Effect of air flow rate on the distribution of energy E in the plane of the maximal mixing intensity in the fermenter with RTS1 (300 rpm) using water (a) and fermentation broth of *A. niger* (b). Air flow rate: 0 vvm (❑—❑), 0.5 vvm (Δ—Δ), 1.0 vvm (■—■), 1.5 vvm (▲—▲), 2.0 vvm (✻—✻). r/R is the ratio of the distance r of the probe from the centre to the radius R of the impeller

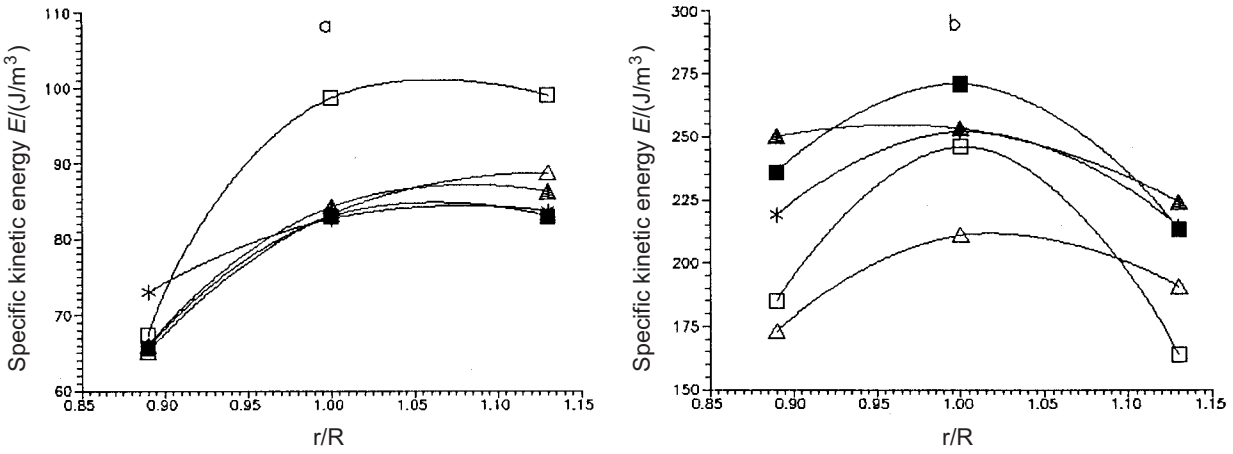

Fig. 3. Effect of air flow rate on the distribution of energy E in the plane of the maximal mixing intensity in the fermenter with ACS1 (500 rpm) using water (a) and fermentation broth of *A. niger* (b). Air flow rate: 0 vvm (❑—❑), 0.5 vvm (Δ—Δ), 1.0 vvm (■—■), 1.5 vvm (▲—▲), 2.0 vvm (✻—✻). r/R is the ratio of the distance r of the probe from the centre to the radius R of the impeller

the lower impeller with the increase in aeration intensity.

The obtained results showed that kinetic energy E measurements could be used to evaluate the mixing conditions in the bioreactor. They also showed that SIMD-f1 might be used as a tool for mixing studies in microbial fermentation broths.

In the second series of experiments, a comparison of the effectiveness of RTS2 and ACS2 on *A. niger* fermentation performance was made by analysing power input characteristics in water and *A. niger* broth. Measurements of power input were carried out in the model equipment using the same bioreactor and mixing systems as in real fermentation of *A. niger*.

The power numbers of RTS2 and ACS2 were estimated in ungassed water and fermentation broth of *A. niger*. According to power number data, RTS2 and ACS2 appeared to be high and low power number stirring systems, respectively. For RTS2, the power number P_O was practically the same ($P_O = 6.8$) in water and *A. niger* fermentation broth. For ACS2, P_O increased approximately 3 times with the increase of biomass concentration and was found to be 0.7 in water, and reached 2.1 in *A. niger* broth with a biomass concentration of 14.2 g/L.

As is seen from the specific power input ε_I [W/kg] measurements in water or *A. niger* broth using RTS2 (Fig. 4), there are only slight differences in ε_I values at the same gas flow rates. In aerated water media, ε_I decreased gradually with an increase in air flow rate. When *A. niger* broth was used, ε_I did not practically change in the range of air flow rate from 0.5 to 2.0 vvm and was approximately twice lower than in ungassed broth. When ACS2 was used, the decrease of specific power input due to the increase of aeration was found lower in *A. niger* broth than in water.

Fig. 5 shows the changes of the specific power input for water and *A. niger* broth at various impeller rotation speeds of RTS2 and ACS2, depending on the gas flow rate. For RTS2, at high stirrer rotation speeds (400–600 rpm), the losses of ε_I due to aeration were higher when compared with losses of ε_I at low rotation speeds (100–200 rpm). For ACS2 at low stirrer rotation speeds (100–300 rpm), aeration did not practically affect the power input values. At high stirrer rotation speeds of ACS2 (500–700 rpm), the fall in ε_I due to aeration was higher than at low rotation speeds of ACS2, but was remarkably lower for ACS2 in comparison with RTS2.

As summarized in Fig. 6, similar power inputs of RTS2 and ACS2 could be achieved at the same agitator

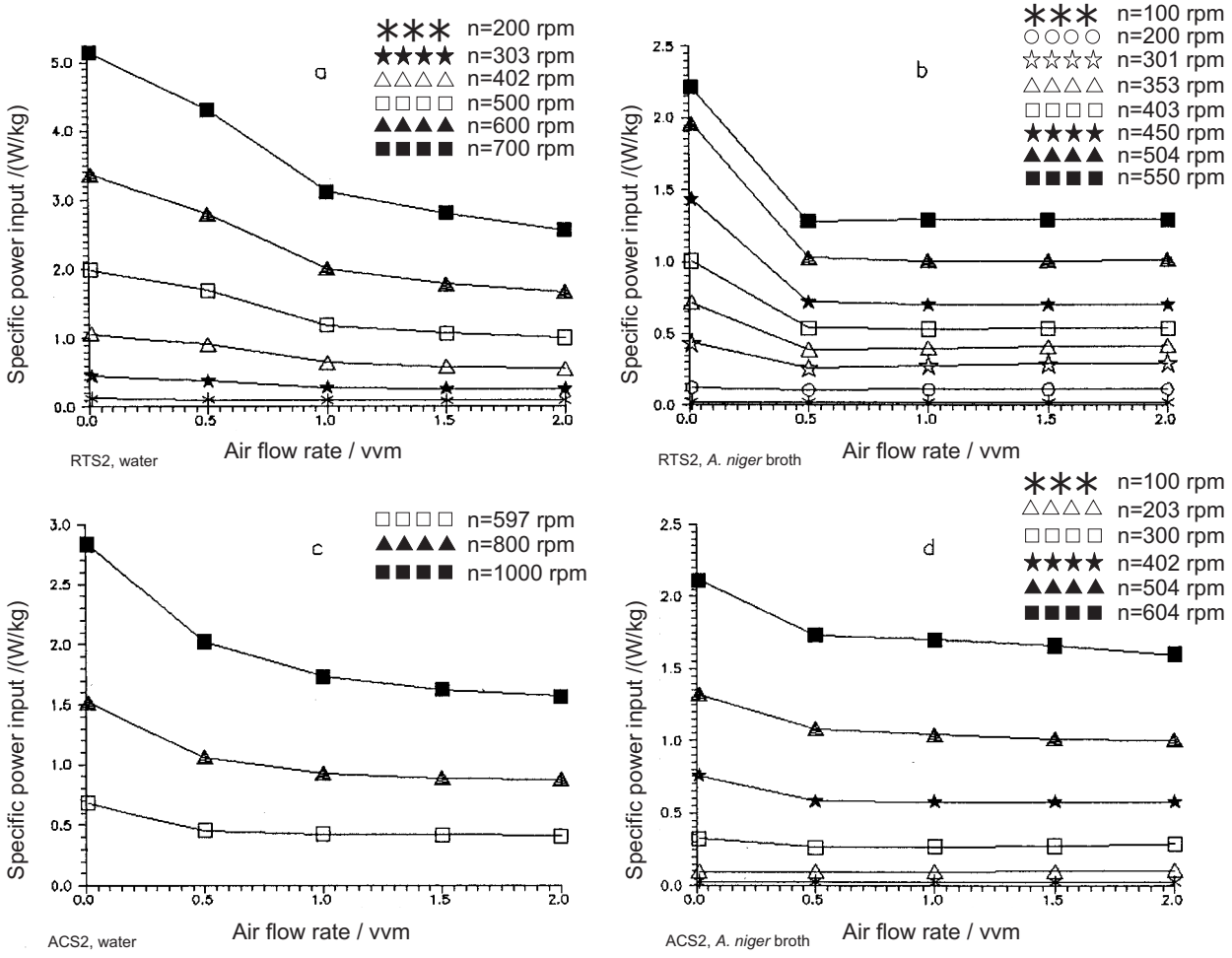

Fig. 4. Dependence of specific power input on the impeller rotation speed of RTS2 (a, b) and ACS2 (c, d) in water (**a, c**) and fermentation broth of *A. niger* (**b, d**) at different gas flow rates

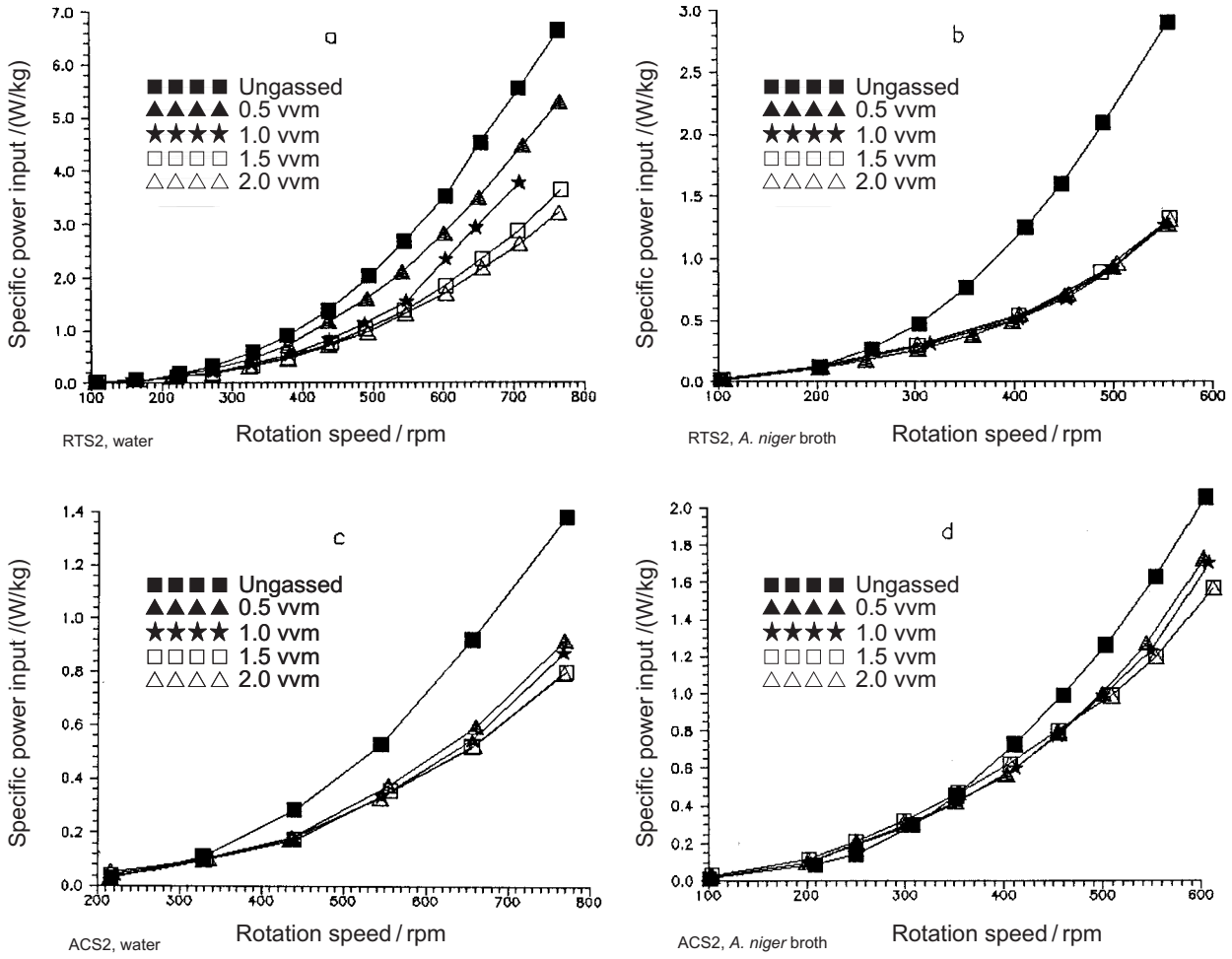

Fig. 5. Dependence of specific power input on the gas flow rate at various impeller rotation speeds using RTS2 (a, b) and ACS2 (c, d) in water (**a, c**) and fermentation broths of *A. niger* (**b, d**)

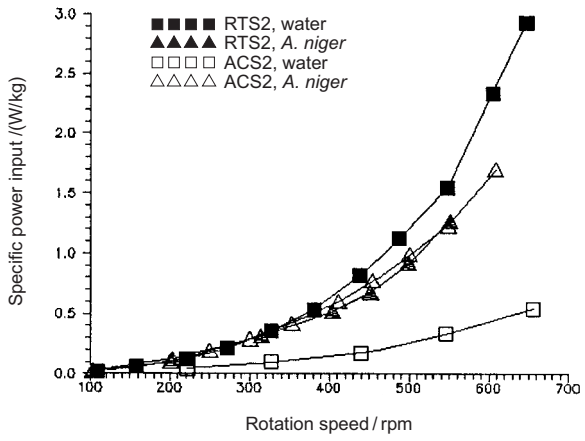

Fig. 6. Changes of the specific power input of RTS2 and ACS2 in water and *A. niger* broth in gassed (1 vvm) media

rotation speeds in gassed *A. niger* broth (14.2 g/L) due to the increase of ε_l in ACS2 at a high *A. niger* biomass concentration.

The effects of gas flow on the power input reduction of RTS2 and ACS2 at 300 and 500 rpm, respectively, are presented in Fig. 7. It is known that higher speeds lead to a greater fall in aerated power. The lower the gassed power/ungassed power (P_g/P) value, the greater the loss of power due to aeration. The losses of power input in ACS2 were smaller than in the case of RTS2 both at low and high agitator rotation speeds. In the case of ACS2, the losses of power input in viscous *A. niger* broth at high agitator rotation speeds were smaller than in water. It is possibly connected with the formation of larger cavities behind the blades of ACS2 in viscous *A. niger* broths. Higher losses of power input in *A. niger* broth in comparison with water were found using RTS2 at lower aeration rates, while at 1.5–2.0 vvm, higher losses in P_g/P were found for water in comparison with *A. niger* broth.

Otherwise, specific power input did not prove to be a criterion for scaling up/down in geometrically dissimilar bioreactors. However, specific power input, expressed in W/kg or kW/m^3, is often considered to be a popular tool to characterize mixing in chemical reactors and bioreactors.

As was shown previously, based on Reynolds number data *(5)*, with the increase of the mycelia concentration, the flow character in biorectors with RTS2 and

Table 1. Mean morphological characteristics of *A. niger* pellets during fermentations when using Rushton turbine stirrer (RTS2) or axial counterflow stirrer (ACS2)

Morphological characteristics	Mixing system	Day of fermentation			
		1st day	3rd day	5th day	7th day
Mean pellet convex area/mm^2	RTS2	8.27 ± 0.47	5.90 ± 0.07	5.61 ± 0.07	6.13 ± 0.06
	ACS2	11.32 ± 1.99	12.37 ± 2.42	14.09 ± 3.28	14.63 ± 1.18
Mean pellet area/mm^2	RTS2	4.45 ± 0.10	2.39 ± 0.21	2.27 ± 0.27	2.36 ± 0.35
	ACS2	5.40 ± 0.83	4.01 ± 0.76	5.07 ± 1.28	4.74 ± 0.36
Mean core convex area/mm^2	RTS2	3.31 ± 0.27	1.41 ± 0.15	1.28 ± 0.29	1.47 ± 0.54
	ACS2	4.72 ± 0.83	3.05 ± 0.84	3.28 ± 0.69	3.04 ± 0.09
Mean core area/mm^2	RTS2	2.83 ± 0.20	1.22 ± 0.12	0.99 ± 0.20	1.22 ± 0.46
	ACS2	3.53 ± 0.56	2.31 ± 0.59	2.47 ± 0.53	2.45 ± 0.23
Mean core convex area/ mean pellet convex area	RTS2	0.40 ± 0.04	0.24 ± 0.05	0.23 ± 0.04	0.25 ± 0.11
	ACS2	0.42 ± 0.01	0.24 ± 0.04	0.24 ± 0.03	0.21 ± 0.01
Mean pellet equivalent diameter/mm	RTS2	2.24 ± 0.12	1.59 ± 0.03	1.66 ± 0.09	1.66 ± 0.01
	ACS2	2.47 ± 0.20	2.12 ± 0.25	2.38 ± 0.34	2.35 ± 0.11
Mean core equivalent diameter/mm	RTS2	1.78 ± 0.12	1.08 ± 0.10	1.04 ± 0.12	1.12 ± 0.20
	ACS2	1.95 ± 0.20	1.55 ± 0.26	1.56 ± 0.20	1.67 ± 0.12
Core circularity	RTS2	1.26 ± 0.12	1.24 ± 0.11	1.22 ± 0.09	1.24 ± 0.05
	ACS2	1.43 ± 0.05	1.35 ± 0.08	1.36 ± 0.15	1.21 ± 0.10
Fullness of annular region / %	RTS2	32.8 ± 1.9	26.4 ± 2.2	29.6 ± 1.3	25.1 ± 3.2
	ACS2	28.6 ± 1.3	18.4 ± 2.0	24.2 ± 3.4	19.8 ± 1.5
Small object area / %	RTS2	41.5 ± 1.9	32.6 ± 7.4	43.8 ± 9.7	46.6 ± 9.4
	ACS2	1.4 ± 0.2	1.8 ± 0.3	1.2 ± 0.2	1.5 ± 0.1

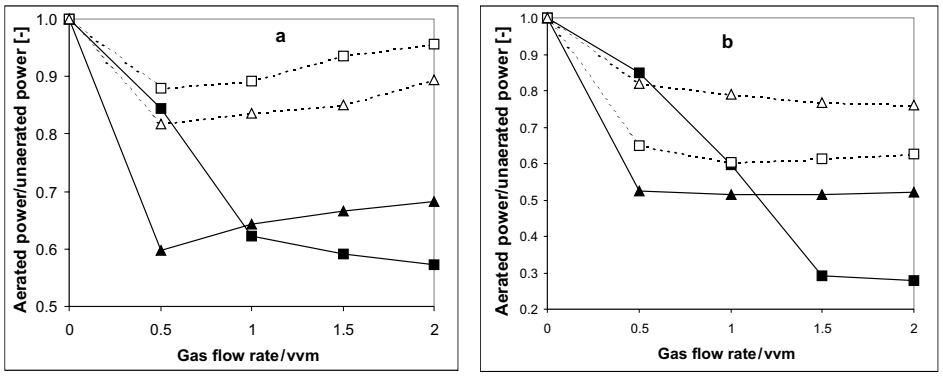

Fig. 7. Effect of aeration rate on the power draw of RTS2 and ACS2 at 300 rpm (a) and 500 rpm (b) in water and *A. niger* broth. Designation: RTS2, water (■—■); RTS2, *A.niger* (▲—▲); ACS2, water (❑—❑); ACS2, *A. niger* (Δ—Δ)

ACS2 changed from turbulent to transient. Possibly, due to a turbulent flow regime in the bioreactor at low biomass concentrations, *A. niger* mycelia seemed to be more sensitive to high impeller rotation speeds at the beginning of fermentation in comparison with the citric acid production phase, when higher agitator rotation speeds are possible.

Due to an increase in the power input of ACS2 with an increase in the biomass concentration and due to a fall in power as a result of the interaction of the air flow rate and impeller rotation speed, an accurate prediction of power input in ACS2 was not possible. The specific power input during the citric acid production phase was approximately 0.7–0.9 W/kg and 0.3–0.6 W/kg for RTS2 and ACS2, respectively. Hence, in *A. niger* fermentation with ACS2, specific power input was approximately twice lower than in the case of RTS2 since higher agitator rotation speeds of ACS2 caused the disruption of mycelia. As *A. niger* is affected by mixing at the beginning of fermentation, equal power inputs would be too low for RTS2 and too high for ACS2. Besides, it was shown in our previous experiments with *Fusarium moniliforme* that the maintenance of an equal specific power input in RTS and ACS did not provide similar profiles of the main fermentation characteristics *(6)*.

In the case of fermentation with ACS2, glucose consumption, biomass growth and citric acid accumulation rates were lower in comparison with the case of RTS2, although similar biomass and citric acid concentrations were achieved at the end of fermentation. The citric acid concentrations at the end of fermentation were 69.8 g/L (RTS2) and 71.9 g/L (ACS2).

During fermentations with RTS2 and ACS2, *A. niger* grew mainly in the form of loose »hairy« pellets. Different morphological features of the pellets were characterized by the image analysis method and are presented in Table 1. The pellet morphology differed depending on the use of RTS2 or ACS2. ACS2 provided the development of a morphology where pellets had larger mean area, convex area and diameter in comparison with the

case of RTS2. During the citric acid synthesis phase, the mean area and convex area of pellets were 2.3–2.7 mm^2 and 5.3–6.4 mm^2, respectively, using RTS2 and 3.5–5.1 mm^2 and 9.4–14.6 mm^2, respectively, using ACS2. The equivalent circ diameter during the citric acid synthesis phase was approximately 1.6–1.8 mm for RTS2 and 2.1–2.4 mm for ACS2. Pellets from the fermentation with ACS2 had substantially lower pellet area/pellet convex area ratios, therefore, pellets in the case of RTS2 were much denser. The roundness data varied both for RTS2 and ACS2, respectively, and the pellet form was very irregular for both stirrer types. ACS2 provided the development of more stretched and irregular pellets.

The cores of pellets in the fermentation with ACS2 had larger diameter, area and convex area in comparison with the cores formed with RTS2, although those differences were not significant. The main differences in pellet morphology when using RTS2 or ACS2 were in the annular region of pellets. The hyphae of pellets in the case of ACS2 were substantially longer, their annular fullness was lower – the hyphae in the outer region of the pellet were less dense.

The morphology of pellets had an effect on the rheological properties of the broth. The viscosity of the final fermentation broths of *A. niger* was measured at the end of the fermentations with RTS2 and ACS2. The data obtained show that *A. niger* broths are highly viscous broths with a pseudoplastic flow behaviour. When described by the power law equation, the consistency index for broth in the case of RTS2 and ACS2 was 5.798 and 3.544, respectively, and the flow behaviour index was 0.192 and 0.255, respectively. In fermentation with RTS2, where smaller and denser pellets were formed but where the concentration of biomass at the end of the process was higher, a higher apparent viscosity of broth was achieved. In fermentation with ACS2, pellets were larger and looser, while broth viscosity was lower in comparison with the case of RTS2. A higher viscosity in the case of RTS2 was obviously due to a higher percentage (32–46 % of the sample measured area) of small objects (separate hyphae and small loose clumps) in the broth, while for ACS2, only 1–2 % of the measured area of mycelia was in diffuse form.

Discussion

Numerous attempts have been made to characterize bioreactors by criteria of similarity and different parameters such as mixing time, power input, oxygen transfer rate and many others. However, they fit more or less correctly in geometrically similar bioreactors.

The culture morphology is of great importance too: operations with bacterial and yeast cells are much simpler in comparison with the mycelia forming ones.

In the present work, two dissimilar bioreactors with different mixing systems with radial or axial impellers were chosen.

It was shown in the previous experiments with gibberellic acid fermentations by *Fusarium moniliforme*, which grows in the form of small clumps and freely dispersed hyphae, that RTS provided a higher concentration of gibberellic acid and the development of a more productive morphology of *F. moniliforme* at an equal specific power input, in comparison with ACS *(6)*. At the same time, for the soft pellets formed by fungus *A. niger* in citric acid fermentation *(8)*, ACS was more effective, as it caused less damage to mycelia, lowered the viscosity of broth and increased the yield of citric acid. Also for *T. viride*, which has a morphology similar to the aforementioned culture, ACS was definitely better, considering the main parameters of cultivation *(15,27)*. One of the possible explanations of this phenomenon could lie in the peculiarities of the interactions of the impeller and the culture liquid. Axial counterflow stirrers had possibly a lower hydrodynamic stress in the region close to the impeller, which could explain the observed differences in cell morphology.

According to Nienow's investigations *(28,29)* for high viscosity mycelial broths, the cavities behind the blades become bigger and much less dependent on aeration rate. It was also seen in the present study that, at the air flow rates from 0.5 to 2 vvm, the specific power input in the *A. niger* broth did not practically change. It is known that, in high viscosity broths, air filled cavities are stabilized and power falls to a constant value more rapidly, not relying on the aeration rate. ACS provided a higher air dispersion at an about twice lower power input, and better bulk flow and mixing dispersed air more effectively, and the losses of the specific power input were lower, which resulted in a higher energy efficiency.

Analysing the *A. niger* morphology, we have observed considerable differences in all main characteristics both for RTS2 and ACS2. In the present fermentation, where RTS2 was used, the pellets were smaller and more compact, but a greater amount of diffuse mycelia was in the broth in comparison with the case of ACS2, thus elevating the viscosity of the broth.

The results of the present work confirm the conclusions made previously by us and many other authors that practically any system (inner constructions of a bioreactor, the producer and the regime of cultivation including mixing) needs its own optimization in order to achieve the final goal – the maximum $Y_{P/S}$ or/and $Y_{X/S}$. For the systems under the present investigation (RTS, ACS), one can apply Nienow's general recommendations *(28,29)*: agitators that are large relative to the bioreactor diameter, with low power numbers (in the case of ACS), can provide better homogenization and air-handling capacity, and higher power inputs under aerated conditions, giving a more effective mass transfer performance. The biotechnological performance of the cultivation and power input data do not correlate in a direct way if the mixing systems (impellers-baffles) are dissimilar.

Principles of process control, especially concerning mixing intensity identification (SIMD–f1 or f3), even stirring regime performance (FAS–5.2) and set of routine fermentation parameters (BIO–2) have been employed as a basis for automation of several technological processes, implemented mainly in the food industry (www.internet.lv/btc).

Conclusions

A special bioreactor design was performed to provide mycelial and other producers sensitive to deformation forces with even mixed cultivation conditions. Axial mixing systems, *e.g.* a counterflow system, were shown to be the most promising in this context.

With the achievement of the critical level of energy E, irreversible changes in morphology obviously occurred. In the case of ACS1, even at high agitator rotation speeds, the energy E values for *A. niger* broth were lower than in the case of RTS1, and better growth and citric acid production were achieved. The analysis of the flow energy distribution by SIMD-f1 or f3 in fermentations of mycelial fungi may be used as a tool for mixing studies not only in model systems, but also in a real fermentation process. However, this methodology could be used only as one of the tools for scaling up and down in geometrically dissimilar systems. For complete solving of scale change procedures, further experiments should be performed.

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Procjena uzgoja *Aspergillus niger* u geometrijski različitim bioreaktorima na osnovi morfoloških analiza

Sažetak

Ispitivani su rast *Aspergillus niger*, proizvodnja limunske kiseline i promjene u morfologiji micelija pod različitim uvjetima miješanja u bioreaktorima s dva tipa miješalica: Rushton turbinske miješalice (RTS1 ili RTS2) i aksijalno protustrujne miješalice (ACS1 i ACS2). Značajke rasta, proizvodnosti i morfologija mijenjali su se s vrstom miješalica i načinom miješanja. U prvoj seriji pokusa analizirane su značajke protoka komine s *A. niger* pod različitim uvjetima miješanja u modelnom bioreaktoru s miješalicama RTS1 i ACS1. Kinetička energija *E* fluktuacije protoka mjerena je u vodi s aeracijom i bez nje, te u fermentacijskim kominama koristeći uređaj za mjerenje intenziteta miješanja (SIMD-f1). Razlika u količini energije *E* na različitim mjestima u bioreaktoru bila je izrazitija u bioreaktoru s RTS1 nego s ACS1. Jako viskozna *A. niger* komina zahtijevala je veće vrijednosti *E* u usporedbi s vodom. Opaženo je da brzina rasta *A. niger* i sinteza limunske kiseline opadaju pri visokim vrijednostima energije *E*, što je očito povezano s utjecajem ireverzibilnog naprezanja na posmik na morfologiju micelija. U drugoj seriji pokusa postignuti su veliki prinosi limunske kiseline primjenom ACS2, pri približno dvostruko manjem utrošku energije u usporedbi s primjenom RTS2. Morfološka karakterizacija peleta *A. niger* provedena je postupkom analize fotografija. Primjenom ACS2 peleti i jezgre bili su veći po opsegu i promjeru, a anularno područje peleta bilo je labavije i »dlakavije« u usporedbi s pokusima s RTS2. Peleti od fermentacije s RTS2 bili su manji, gušći, s kraćim hifama u anularnom području peleta, a komina je sadržavala veći postotak difuznih micelija. Studije utroška snage primjenom RTS2 i ACS2 provedene su pri različitim brzinama okretaja miješalica i protoka zraka u vodi i u komini s *A. niger*. RTS2 ima sustav miješanja s velikim utroškom snage (P_0 = 6,8) koji se praktički ne mijenja ako se promijeni medij. Za ACS2 se P_0 povećava približno tri puta, od 0,68 u vodi do 2,11 u viskoznoj komini s *A. niger*. Analiziran je utjecaj miješanja i jačine aeracije na utrošak energije primjenom RTS2 i ACS2. ACS2 bio je djelotvorniji jer se aeracijom gubilo puno manje energije.