Effect of Pulsing Mixing Interruptions on the *Aspergillus niger* Morphology and Citric Acid Production

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The effect of short pulsing mixing interruptions on the *Aspergillus niger R3* morphology and fermentation performance was studied in 5 l agitated fermenters with double Rushton turbine impellers. A stable increase in the mycelia concentration and higher biomass concentrations at the end of the process were achieved in two continuous mixing fermentations, while in two fermentations with mixing interruptions biomass accumulation level was significantly lower. The highest citric acid concentration, in comparison with continuous mixing fermentations, was obtained in the pulsed on/off mixing fermentation with gas blending, and the pulsed mixing fermentation without gas blending. Small pellets (with about 0.6 – 0.8 mm mean equivalent diameter) developed in continuous mixing fermentations. The largest mean equivalent pellet diameter (approximately 0.9 – 1.1 mm) and the longest hyphae in the outer zone of a pellet were observed in the most productive fermentation with mixing oscillations accompanied by gas blending.

Keywords:
Pulsing mixing interruptions, *Aspergillus niger*, citric acid, morphology

Introduction

In *A. niger* fermentations, where mixing and aeration conditions are crucial for high citric acid production, the cessation of oxygen supply could cause decreased product yields. Reports are available concerning the negative effect of the interruptions of oxygen supply on citric acid production.[1,2]

Oxygen has been shown by *Kubicek* et al.[1] to be a direct regulator of citric acid accumulation, since a response is obtained within few minutes after altering the oxygen supply. Interruptions in aeration have been shown to cause the impairment of the respiratory mechanisms. The interruptions of aeration for 20 min by disconnecting the impeller have been found to irreversibly alter, both, citric acid synthesis and the respiration of *Aspergillus niger*, although, the recovery of tropophase cultures has been observed. The investigations of *Dawson* et al.[2] have shown the possibility of recovery of, both, tropophase and idiophase of *A. niger* cultures after air supply and impeller motion interruptions in citric acid fermentations, if the dissolved oxygen level remains above the critical value.

The fall of the dissolved oxygen concentration below 20 % of saturation has no pronounced effect on the performance of the culture.[2] If the dissolved oxygen concentration falls to zero, both, mycelial growth and citric acid production are inhibited. On resuming air supply, the citric acid production rate is regained to a level similar to that observed prior to the interruption. Slight inhibitory effects on citric acid production are observed when the interruptions take place during the early stages of fermentation, but these effects are rapidly overcome. When interruptions occur later in the fermentation, i.e. during the idiophase, no effect is observed. The latter result appears to be in contrast to that reported by *Kubicek* et al.,[1] who has stated that idiophase cultures fail to recover from temporary interruptions of air supply. For the experiments, where the air supply is interrupted for 20-min periods, DO never falls below 20 % of saturation. As it has been concluded by *Dawson* et al.,[2] if DO remains above the critical value, no real effect would be apparent. Pure oxygen rather than air has been shown to lead to increased product yields.[3]

Kubicek et al.[1] demonstrated that short-term changes in DO produce immediate irreversible changes in the rate of product formation. It has been clearly shown that citric acid accumulation is favoured by increasing DO of the fermentation medium. The effect of the cessation of aeration on citric acid production depends in part on the phase at which the interruption occurs and on the duration of this interruption. Oxygen has been demonstrated to act as a direct regulator of citric acid accumulation, since a response is obtained within only several minutes after altering the oxygen supply. Changes in citric acid production are accompanied by

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changes in the oxygen uptake rate, whereas carbon dioxide formation remains relatively unaffected.

In submerged fermentations, *A. niger* can grow into a variety of morphological forms, varying from filamentous ones to pellets. The rheological characteristics of filamentous forms have been shown to be highly non-Newtonian and viscous, limiting mixing and aeration.[4] The broth with a pellet morphology is characterized by lower viscosities and better mass transfer properties.

The pellet structure can vary from loose and irregular to compact and spherical. Agitation has been shown to influence the structure and survival of pellets. Fluffy loose pellets have been found to be preferred for citric acid production.[5] However, increasing attention is focused on filamentous fermentations with a dispersed growth form, as a more intensive supply of nutrients and oxygen could be attained.[6] The inoculum spore concentration and medium pH are also of considerable importance in the pellet structure.[7]

Interruptions of agitation during laboratory fermentation in flasks result in about a 20 % decrease in final citric acid yields; however, a prolonged limitation of gas exchange brings about a marked drop in citric acid production in flasks even under uninterrupted culture agitation.[8] Even a short interruption affects dramatically the biosynthetic activity of *A. niger*.[9]

The design of fermenter mixing systems has been shown to influence significantly the productivity and morphology of *A. niger*, sensitive to deformation forces of stirrers.[10]

The aim of the present investigation was to study the effect of short pulsing mixing interruptions on the citric acid fermentation performance and *A. niger* morphology.

Experimental

Microorganism and culture conditions

The citric acid producing fungus *A. niger* R3 spores from the microbial strain collection of the University of Latvia were used.[11] The initial spore concentration of the inoculum was approximately 1.2×10^7 spores ml^{-1}. The inoculum was grown in a shaker incubator (Controlled Environment Incubator Shaker, New Brunswick Scientific Co., Edison, U.S.A.) in 2 l flasks containing 600 ml of the medium for 26 h for the first run (fermentations P_1 and P_2) or 29 h (3 h more due to technical reasons) for the second run (fermentations P_3 and P_4) at the temperature 32 °C and stirred at n = 220 min^{-1}. The inocula and synthetic fermentation (glucose 120 g l^{-1}) media composition was as described earlier.[12] Fermentation media (4.0 l) were inoculated with 500 ml of the mycelia suspension from the shake flasks.

The medium temperature in fermenters was controlled at 32 ± 0.3 °C. Fermentation time was 142 h (fermentations P_1 and P_2) and 158 h (fermentations P_3 and P_4).

Aeration rate was increased from 0.5 to 1.0 vvm during the first day, and the impeller rotational speed was changed from n = 300 min^{-1} on the first day to n = 500 min^{-1} on the second day and to n = 700 min^{-1} up to the end of fermentations (Table 1).

Table 1 – *Impeller rotational speed and air flow rate during A. niger fermentations*

Fermentation number	Agitation regime		Aeration regime		Gas blending time, h	Dissolved oxygen / %
	impeller rotational speed, n / min^{-1}	time / h	air flow rate Q / l l^{-1} min^{-1}	time / h		
P_1, Continuous mixing	300	0–21.5	0.5	0–20		100…20
	500	21.5–51.5	1	20–142		40–50
	700	51.5–142				70–90
P_2, Pulsing mixing with interruptions, gas blending	300	0–21.5	0.5	0–20	8.7–57	100…30
	500	21.5–51.5	1	20–142		30–50
	700	51.5–142				50–90
P_3, Continuous mixing	300	0–18.3	0.5	0–12		100…20
	500	18.3–48.5	1	12–158		40–50
	700	48.5–158				60–70
P_4, Pulsing mixing with interruptions	300	0–18.3	0.5	0–12		100…10
	500	18.3–48.5	1	12–158		10–20
	700	48.5–158				30–50

Fermentation equipment

Fermentations were carried out in 5 l fermenters (Electrolab Ltd., Scientific Instruments Manufacturers, U.K.) with a 4.5 l working volume. The diameter and height of the fermenters were 160 and 310 mm, respectively. The bottom of the vessels was flat. The fermenters had 4 baffles (width 15 mm). The height of the 4.5 l fermentation vessel was 225 mm. Double tier Rushton turbine impellers were used. Impellers consisted of a disc (31 mm in diameter) with 6 blades (28 x 16 mm) and with a total impeller diameter of 83 mm. The distance between the impellers' middle plane was 100 mm, and that between the middle plane of the lower impeller and the bottom of the fermenter was 33 mm.

Dissolved oxygen and pH measurements

For dissolved oxygen measurements, polarographic INGOLD electrodes (Mettler Toledo Ltd., Leicester, U.K.), were used. In fermentation P_2 with mixing interruptions, the dissolved oxygen level was maintained above 40 % of saturation by mixing oxygen and air using a gas blender (GA Platon Ltd., Basingstoke, U.K.). pH was measured using glass INGOLD 465-35-K9 electrodes (Mettler Toledo Ltd., Leicester, U.K.).

Characterization methods

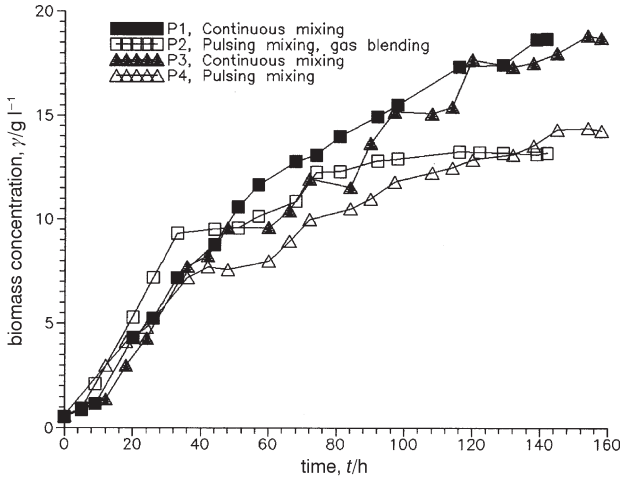
Inlet and exit gas analysis were provided by a VG Mass Spectrometer (VG MM8-80 Mass Spectrometer, VG Gas Analysis Systems Ltd., Middlewich, U.K.), interfaced with a SETCON process management and control system (Aspen Tech Inc., Houston, USA).

Citric acid and glucose assay was conducted by high pressure liquid chromatography (HPLC), and dry mass estimation was as shown by *Paul* et al.[13] Image analysis of the *A. niger* pellet morphology was carried out using the method and software developed by Cox and Thomas.[14,15]

Results

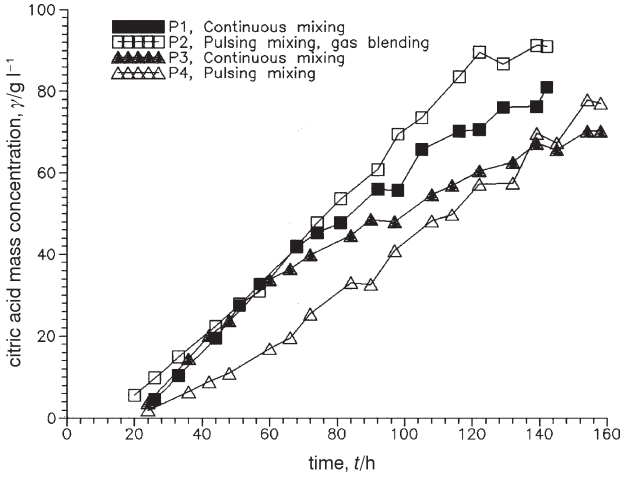
Four fermentations, two of them at a continuous impeller speed (P_1 and P_3) and two at a pulsing (on/off 30/30 s) impeller speed (P_2 and P_4), were carried out by using the same impeller rotational speed profiles, starting with 300 min^{-1}, then changing to 500 min^{-1} on the second day and to 700 min^{-1} on the third day (Table 1). In fermentations with mixing interruptions, one of them (P_2) had the gas blending during 8.7 – 57 h, while in the other (P_4), gas blending was not used. The pH course during the processes was similar for all fermentations, and after the initial value 5.6 in the medium, it fell to approximately 3.1 – 3.2 after inoculation and was in the range from 1.75 to 2.10 during the processes.

As it is seen from Fig. 1, mixing pulse with interruptions favoured the *A. niger* biomass growth during the first 20 – 30 h of fermentations P_2 and P_4. After the period of favoured growth, biomass accumulation was clearly inhibited in both fermentations with mixing oscillations. In fermentation P_2 with pulse mixing, where DO was near 30 – 50 % by using gas blending during the intensive growth phase, a reduction in growth rate was observed earlier (approximately 36 – 60 h). In fermentation P_4, where DO was near 10 – 20 % during the first two days, the inhibitory effect of the impeller pulse on biomass growth was observed later (in approximately 50 – 70 h). Considerably lower biomass concentrations (13.20 and 14.28 g l^{-1} for fermentations P_2 and P_4, respectively) were obtained at the end of fermentations with pulse mixing in comparison with P_1 and P_3, where continuous mixing was kept throughout the fermentation, and biomass concentrations were 18.70 and 18.73 g l^{-1}, respectively.



F i g . 1 – *A. niger biomass growth during fermentations P_1, P_2, P_3 and P_4*

The highest glucose consumption and citric acid production rates were achieved in fermentation P2 with pulse mixing, where a high DO level (30 – 50 % during the first 3 days) was maintained by gas blending during 8.7 – 57 h, and the maximum citric acid concentration was achieved already on the 5th day of fermentation (Fig. 2). The lowest rates of glucose consumption, biomass accumulation and citric acid accumulation were observed in mixing pulse fermentation P_4 having a low dissolved oxygen level (10 – 20 %) during the first two days of the process, yet an increase in the citric acid accumulation rate was observed during the last days of fermentation, when a DO of 30 – 50 % was main-



F i g . 2 – *Citric acid accumulation during A. niger fermentations P_1, P_2, P_3 and P_4*

tained by a high impeller rotational speed, thus indicating the restoration of the culture from the oxygen limit in the early phase.

As is summarized in Table 2, mixing pulse in both fermentations, with gas blending (P_2) or without it (P_4), increased the yield of citric acid from biomass ($Y_{P/X}$) and decreased the yield of biomass from glucose ($Y_{X/S}$) in comparison with continuous mixing fermentations P_1 and P_3.

T a b l e 2 – *Yield coefficients in citric acid fermentations by A. niger in conditions of continuous or pulsing mixing with interruptions*

Fermentation number	Characterization of fermentation	$Y_{X/S}$	$Y_{P/S}$	$Y_{P/X}$
P_1	Continuous mixing	0.166	0.739	4.459
P_2	Pulsing mixing with interruptions, gas blending	0.106	0.758	7.182
P_3	Continuous mixing	0.162	0.627	3.878
P_4	Pulsing mixing with interruptions	0.118	0.667	5.633

The oxygen uptake (OUR) and carbon dioxide production (CPR) rates in fermentations P_1 and P_3 with continuous stirring were approximately 9–10 mmol $l^{-1}\cdot h^{-1}$ for OUR and 5–8 mmol $l^{-1}\cdot h^{-1}$ for CPR during the first two days, with a slow decrease to approximately 5–8 mmol $l^{-1}\cdot h^{-1}$ for OUR and 3–5 mmol $l^{-1}\cdot h^{-1}$ for CPR during the citric acid production phase. The respiratory quotient (RQ) for these fermentations increased during the intensive citric acid production phase from approximately 0.5 to 0.8 till the end of fermentation.

Fermentation P_2 with the impeller on/off mode and gas blending reached the highest oxygen uptake rate (20–25 mmol $l^{-1}\cdot h^{-1}$) during the biomass production on the first two days, but starting with the 3rd day, OUR and CPR were similar to the case of fermentation P_1. Lower CPR, similar OUR and, as a result, lower RQ (0.5–0.6) values were observed during the intensive citric acid production on the 3rd – 5th day of fermentation P_2, when a substantially higher citric acid accumulation rate was observed in comparison with the cases of continuous mixing. A marked increase in RQ (as a result of the decrease of OUR and the increase of CPR) at the very last stage of this fermentation took place due to practically consumed glucose, resulting in the slow-down of citric acid accumulation.

In the case of impeller on/off oscillations and low DO levels (fermentation P_4 without gas blending), the lowest OUR and CPR values were observed during the first two days of fermentation, in comparison with other fermentations. After the increase of the impeller rotational speed to 700 rpm, OUR and CPR values were similar to other fermentations.

As pellet inocula were used for inoculation, mainly pelleted growth developed throughout the fermentations (Tables 3 and 4). In fermentations P_1 and P_3 with continuous mixing during the first day of the process, when the impeller rotational speed 300 min^{-1} was maintained, the mean equivalent diameter of pellets increased from approximately 0.35 to 0.60 mm, and the hyphae in the outer zone of pellets were very short. After the increase of the impeller rotational speed to 500 min^{-1}, both, pellet cores and hyphae in the outer zone increased during this period, and the mean equivalent diameters of cores and pellets reached approximately 0.5 – 0.7 mm and 0.6 – 0.8 mm, respectively. When the impeller rotational speed was changed to 700 min^{-1}, the pellets and their core sizes as well as the fullness in the outer region changed little, with a slight decrease up to the end of fermentation. During the citric acid synthesis phase, the mean pellet projected area was in the range of approximately 0.2 – 0.4 mm^2.

In fermentation P_2 (pulse mixing with gas blending), larger pellets (up to the mean equivalent pellet diameter approximately 0.85 mm) with short hyphae in the outer region developed during the first day. After the increase of the impeller rotational speed up to 500 min^{-1}, the pellet size slightly decreased to approximately 0.7 mm during the second day. When the impeller rotational speed was increased to 700 min^{-1}, the core and pellet mean equivalent diameter increased, varying in the ranges 0.8 – 1.0 mm and 0.9 – 1.2 mm, respectively. The longest hyphae in the outer region were found in

Table 3 – *Time course of the pellet total area (mm²) during A. niger fermentations P_1, P_2, P_3 and P_4*

Run 1			Run 2		
Time / h	P_1, continuous mixing	P_2, pulsing mixing with interruptions, gas blending	Time / h	P_3, continuous mixing	P_4, pulsing mixing with interruptions
0	0.112±0.008	0.076±0.008	0	0.043±0.005	0.122±0.005
5	0.073±0.007	0.105±0.007	12	0.141±0.020	0.134±0.016
9	0.203±0.019	0.154±0.027	18	0.116±0.023	0.164±0.030
20	0.266±0.044	0.424±0.060	20	0.215±0.029	0.133±0.021
22	0.289±0.036	0.577±0.063	24	0.292±0.047	0.177±0.028
26	0.487±0.080	0.689±0.083	36	0.238±0.042	0.149±0.023
33	0.499±0.095	0.521±0.083	42	0.374±0.075	0.308±0.056
44	0.519±0.091	0.421±0.077	48	0.508±0.099	0.264±0.067
51	0.337±0.047	0.602±0.060	60	0.268±0.048	0.157±0.034
52	0.338±0.058	0.547±0.110	66	0.280±0.045	0.309±0.053
57	0.562±0.110	0.523±0.073	72	0.392±0.073	0.156±0.024
68	0.424±0.093	0.653±0.085	84	0.272±0.051	0.214±0.048
74	0.383±0.065	1.015±0.178	90	0.170±0.023	0.155±0.039
81	0.490±0.087	0.657±0.117	97	0.274±0.055	0.222±0.047
92	0.268±0.042	0.567±0.095	108	0.294±0.065	0.148±0.031
98	0.279±0.052	0.731±0.155	114	0.206±0.025	0.199±0.045
105	0.480±0.094	1.076±0.240	122	0.164±0.024	0.171±0.031
116	0.321±0.065	1.223±0.280	132	0.289±0.061	0.277±0.057
122	0.397±0.060	0.864±0.178	139	0.235±0.055	0.309±0.085
129	0.313±0.073	0.657±0.099	145	0.268±0.037	0.234±0.062
139	0.175±0.030	0.714±0.133	154	0.219±0.036	0.229±0.037
			158	0.153±0.017	0.185±0.026

this most productive fermentation during the intensive citric acid production phase, although a similar density of hyphae (measured as annular fullness) in the outer pellet zone was achieved in comparison with the cases of other fermentations. The mean pellet projected area during the late phase of citric acid production was approximately 0.7 – 1.1 mm².

If the on/off mixing pulse was accompanied by a lower, insufficient DO level during the first two days in fermentation P_4, the smallest pellets (mean equivalent diameter approximately 0.4 – 0.6 mm, and mean projected area approximately 0.15 – 0.30 mm²) with very short hyphae in the outer region were observed throughout the whole fermentation. Slightly longer hyphae in the outer region developed at the final stage of fermentation, when the citric acid accumulation rate was higher in comparison with the main course.

Similar core/pellet mean projected convex area ratios (approximately 0.6 – 0.7) and annular fullness values (approximately 50 – 60 %) were observed in all fermentations during the intensive citric acid production phase (Table 5).

Oxygen transfer rate (OTR) and oxygen transfer coefficient (k_La) changes were analyzed in bioreactors at the end of fermentations in the final fermentation broths of *A. niger* at the impeller rotational speeds from 400 to 700 min^{-1} and the aeration rates 0.5 and 1.0 l l^{-1} min^{-1}. In fermentations P_1 and P_3 with continuous mixing, where a high biomass level (18.7 g l^{-1}) was accumulated, the OTR at 1 l l^{-1} min^{-1} was lower in comparison with the case of fermentations P_2 and P_4 with mixing oscillations, where the mass concentrations of biomass at the end of the process were 13.20 and 14.28 g l^{-1}, respectively. In fermentations with continuous mixing, the OTR in fermentation P_1 increased slightly

Table 4 – *Time course of the pellet core area (mm²) during A. niger fermentations P_1, P_2, P_3 and P_4*

Run 1			Run 2		
Time / h	P_1, continuous mixing	P_2, pulsing mixing with interruptions, gas blending	Time / h	P_3, continuous mixing	P_4, pulsing mixing with interruptions
0	0.105±0.007	0.074±0.007	0	0.042±0.005	0.117±0.011
5	0.072±0.007	0.104±0.007	12	0.131±0.017	0.121±0.015
9	0.180±0.017	0.093±0.015	18	0.114±0.023	0.136±0.022
20	0.252±0.042	0.390±0.054	20	0.173±0.020	0.119±0.012
22	0.239±0.029	0.542±0.057	24	0.232±0.039	0.147±0.020
26	0.299±0.036	0.601±0.071	36	0.230±0.040	0.129±0.017
33	0.318±0.056	0.489±0.075	42	0.311±0.059	0.268±0.051
44	0.380±0.067	0.408±0.070	48	0.381±0.062	0.194±0.037
51	0.260±0.032	0.390±0.054	60	0.267±0.048	0.151±0.031
52	0.305±0.049	0.425±0.068	66	0.247±0.036	0.252±0.056
57	0.341±0.052	0.467±0.064	72	0.308±0.053	0.146±0.021
68	0.297±0.051	0.583±0.071	84	0.251±0.044	0.205±0.045
74	0.328±0.046	0.882±0.153	90	0.169±0.023	0.148±0.036
81	0.383±0.066	0.519±0.089	97	0.235±0.040	0.203±0.042
92	0.190±0.018	0.490±0.073	108	0.219±0.029	0.139±0.025
98	0.209±0.037	0.455±0.081	114	0.199±0.022	0.173±0.036
105	0.314±0.065	0.672±0.145	122	0.159±0.022	0.154±0.023
116	0.248±0.036	0.893±0.189	132	0.280±0.055	0.238±0.046
122	0.259±0.035	0.581±0.105	139	0.222±0.050	0.271±0.073
129	0.228±0.047	0.542±0.080	145	0.259±0.035	0.206±0.049
139	0.114±0.013	0.495±0.070	154	0.203±0.032	0.178±0.024
			158	0.152±0.017	0.159±0.019

from 4.6 mmol O_2 $l^{-1}{\cdot}h^{-1}$ at 400 min^{-1} to 5.0 mmol O_2 $l^{-1}{\cdot}h^{-1}$ at 700 min^{-1}, and from 3.9 mmol O_2 $l^{-1}{\cdot}h^{-1}$, to 4.1 mmol O_2 $l^{-1}{\cdot}h^{-1}$, at the same impeller rotational speeds. The OTR values for *A. niger* broths obtained with mixing oscillations varied from 4.9 to 5.5 mmol O_2 $l^{-1}{\cdot}h^{-1}$ and from 5.3 to 5.8 mmol O_2 $l^{-1}{\cdot}h^{-1}$ for P_2 and P_4, respectively, at the same impeller rotational speeds.

The oxygen transfer coefficient was lower for the continuous mixing fermentation P_1 and, with increasing impeller rotational speed from 400 to 700 min^{-1}, increased twice from 32 to 60 h^{-1}. In fermentations P_2 and P_4, the k_La values in final broths were higher and increased from 44 to 89 h^{-1} for P_2 and from 32 to 98 h^{-1} for P_4, increasing the rotational speed from 400 to 700 min^{-1}, thus indicating better oxygen transfer properties of these broths.

Discussion

In *A. niger* fermentations, dissolved oxygen tension, maintained by effective mixing and aeration conditions, is a prerequisite for good citric acid production. In the present study, the effect of a short mixing pulse with speed interruptions (on/off 30/30 s) in citric acid fermentation by *A. niger* was investigated. The current study showed a tendency of a lower biomass accumulation level and favoured the acid accumulation with a short mixing on/off pulse, if a sufficient dissolved oxygen level was maintained by gas blending during the early phase at a lower impeller rotational speed. In both fermentations with mixing pulses, a higher rate of biomass accumulation was observed during the early stage. As it has been shown earlier, due to the turbulent flow regime at low biomass concentrations, the mycelia of *A. niger* were more sensitive

Table 5 – *Time course of the pellet annular fullness (%) during A. niger fermentations P_1, P_2, P_3 and P_4*

Run 1			Run 2		
Time / h	P_1, continuous mixing	P_2, pulsing mixing with interruptions, gas blending	Time / h	P_3, continuous mixing	P_4, pulsing mixing with interruptions
0	77.5±9.0	89.0±1.8	0	85.9±1.8	84.5±1.6
5	87.0±1.6	88.1±1.7	12	63.1±3.0	55.8±2.5
9	69.9±3.3	74.5±2.2	18	73.2±3.3	61.6±2.9
20	63.0±3.9	64.1±3.2	20	53.4±2.6	59.3±2.5
22	52.3±2.6	63.4±4.1	24	60.1±3.8	54.4±2.4
26	46.6±3.5	61.0±3.9	36	61.4±3.4	61.5±3.2
33	47.4±4.1	71.7±3.9	42	63.0±3.7	58.1±2.6
44	43.8±4.0	52.5±3.2	48	51.3±3.0	62.2±2.9
51	46.7±3.5	64.1±3.8	60	45.2±3.7	60.7±3.1
52	56.0±8.1	71.9±4.1	66	50.5±3.1	49.9±2.6
57	53.0±4.0	54.1±4.2	72	66.6±3.9	70.3±3.0
68	45.7±4.9	61.4±3.6	84	49.1±3.0	52.5±3.0
74	51.8±3.6	66.7±4.2	90	55.2±3.0	71.3±3.3
81	57.9±4.1	52.0±4.0	97	50.5±3.0	53.1±3.1
92	57.1±3.7	53.1±4.8	108	62.4±3.7	63.5±3.4
98	52.9±3.2	52.1±5.2	114	52.5±2.6	49.8±2.9
105	48.6±3.2	52.6±5.0	122	55.6±2.8	53.8±3.3
116	45.3±3.9	61.9±6.0	132	58.5±2.6	51.6±3.2
122	59.6±4.0	62.7±5.2	139	69.1±3.4	46.0±3.6
129	60.0±3.3	52.5±3.2	145	55.2±2.4	47.6±3.4
139	60.1±3.0	60.4±9.6	154	61.6±2.8	41.7±2.7
			158	66.8±2.5	48.4±3.1

to high impeller rotational speeds at the beginning of fermentation in comparison with the citric acid accumulation phase, when much higher impeller rotational speeds were easily tolerated.[12] Less turbulent mixing conditions due to the impeller pulse were possibly the reason for favoured growth of *A. niger*, especially in fermentation P2 with sufficient dissolved oxygen conditions. The inhibitory effect of mixing pulses on biomass accumulation was observed after the fast growth stage and lasted for about 24 h for both fermentations. After this period, the recovery of biomass accumulation took place, yet a markedly lower biomass concentration (24 – 29 % less) was obtained at the end of fermentations with mixing pulses in comparison with continuous mixing fermentations. Although, the low DO level in fermentation P_4 with mixing pulses slowed down the biomass accumulation, similar final biomass concentrations were achieved in both fermentations with pulsing mixing interruptions, although, the citric acid yield was decreased in P_4 due to the low level of DO at early stages.

Conclusion

Aspergillus niger populations are sensitive to short pulsing mixing interruptions at the first stages of fermentations, if a high enough dissolved oxygen level could not be maintained by sufficient aeration. If additional oxygen supply by gas blending is provided in fermentation with mixing interruptions, more productive pellets with longer hyphae in outer zone are formed. During the production phase, when a high enough biomass concentration is achieved, short mixing on/off pulses lead to a higher culture productivity, and higher citric acid concentrations are attained at lower biomass levels.

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Symbols

DO – dissolved oxygen, %

$Y_{P/X}$ – yield of citric acid related to biomass

$Y_{X/S}$ – yield of biomass from glucose

$Y_{P/S}$ – yield of citric acid from glucose

OUR – oxygen uptake rate, mmol l^{-1} h^{-1}

CPR – carbon dioxide production rate, mmol l^{-1} h^{-1}

OTR – oxygen transfer rate

k_La – oxygen transfer coefficient, h^{-1}

RQ – respiratory quotient

HPLC – high pressure liquid chromatography

n – rotation speed, min^{-1}

Q – air flow rate, l l^{-1} min^{-1}

γ – mass concentration, g l^{-1}

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