J Ind Microbiol Biotechnol (2011) 38:565–571
DOI 10.1007/s10295-010-0804-8

ORIGINAL PAPER

Production of L-lactic acid by *Rhizopus oryzae* using semicontinuous fermentation in bioreactor

Xuefeng Wu · Shaotong Jiang · Mo Liu · Lijun Pan · Zhi Zheng · Shuizhong Luo

Received: 12 March 2010 / Accepted: 31 July 2010 / Published online: 9 September 2010

Abstract Semicontinuous fermentation using pellets of *Rhizopus oryzae* has been recognized as a promising technology for L-lactic acid production. In this work, semicontinuous fermentation of *R. oryzae* AS 3.819 for L-lactic acid production has been developed with high L-lactic acid yield and volumetric productivity. The effects of factors such as inoculations, $CaCO_3$ addition time, and temperature on L-lactic acid yield and *R. oryzae* morphology were researched in detail. The results showed that optimal fermentation conditions for the first cycle were: inoculation with 4% spore suspension, $CaCO_3$ added to the culture medium at the beginning of culture, and culture temperature of 32–34°C. In orthogonal experiments, high L-lactic acid yield was achieved when the feeding medium was (g/l): glucose, 100; $(NH_4)_2SO_4$, 2; KH_2PO_4, 0.1; $ZnSO_4{\cdot}7H_2O$, 0.33; $MgSO_4{\cdot}7H_2O$, 0.15; $CaCO_3$, 50. Twenty cycles of semicontinuous fermentation were carried out in flask culture. L-lactic acid yield was 78.75% for the first cycle and 80–90% for the repeated cycles; the activities of lactate dehydrogenases (LDH) were 7.2–9.2 U/mg; fermentation was completed in 24 h for each repeated cycle. In a 7-l magnetically stirred fermentor, semicontinuous fermentation lasted for 25 cycles using pellets of *R. oryzae* AS 3.819 under the optimal conditions determined from flask cultures. The final L-lactic acid concentration (LLAC) reached 103.7 g/l, and the volumetric productivity was 2.16 g/(l·h) for the first cycle; in the following 19 repeated cycles, the final LLAC reached 81–95 g/l, and the volumetric productivities were 3.40–3.85 g/(l·h).

Keywords L-lactic acid · *Rhizopus oryzae* · Semicontinuous fermentation · Pellets

X. Wu (✉) · S. Jiang (✉) · M. Liu · L. Pan · Z. Zheng · S. Luo
School of Biotechnology and Food Engineering,
The Key Laboratory for Agricultural Products Processing of Anhui Province, Hefei University of Technology,
No. 193 Tunxi Road, 230009 Hefei City,
Anhui Province, People's Republic of China
e-mail: applewuxf@hotmail.com

S. Jiang
e-mail: jianghsaotong@yahoo.com.cn

Introduction

L-lactic acid is a commonly occurring organic acid that is valuable due to its wide use in food-related industries and its potential use in production of biodegradable polylactate polymers. The increased use of L-lactic acid in exciting applications and its potential use in biodegradable plastics has made L-lactic acid production an attractive investment. L-lactic acid is produced by bacteria and fungi. Fungal *Rhizopus* species generate L-lactic acid as a sole isomer of lactic acid and have been recognized as promising candidates for L-lactic acid production [1–3].

Filamentous fungi in submerged culture grow into loose mycelial clumps or pellets. The mycelial morphology is influenced by a number of factors such as strain, medium composition, inoculum size, pH, mechanical forces, dissolved oxygen tension, temperature, medium viscosity, etc. Morphology influences the rate of microorganism growth and product formation. In case of L-lactic production by *Rhizopus arrhizus*, lactic acid volumetric productivity and yield decreased to 1.63 kg/(m^3·h) and 62.6%, respectively, when the morphology changed to pellets [4]. However, *Rhizopus oryzae* in pellet form exhibited higher lactic acid yield than in clump form [5]. Therefore, researchers studied the effect of environmental factors such as nitrogen and glucose concentration on fungi growth to obtain high biomass

concentrations in the form of smooth pellets [6], which were found to be beneficial for repeated cycle fermentation.

Recently, semicontinuous fermentation for L-lactic acid production using *Rhizopus* species has been developed. Semicontinuous fermentation by *R. oryzae* can be separated into the cell growth stage and the production formation stage. In the cell growth stage, *R. oryzae* grows into uniform pellets, in the presence of carbon source, nitrogen source, and other ions. In the production formation stage, fermentation is carried out using the existing uniform pellets; with less ions and nitrogen source, higher L-lactic acid yield can be reached in shorter time. Yu et al. used floc-form *R. oryzae* to produce L-lactic acid by semicontinuous fermentation; for the first four cycles the volumetric productivity and yield were 4.03 g/(l·h) and 0.90, respectively, in 28 h, but volumetric productivity decreased to 3.44 g/(l·h) in 32 h in cycle 5 and cycle 6 [7]. Yin et al. used small mycelia pellets of *R. oryzae* to produce L-lactic acid for nine cycles over 14 days, and the average volumetric productivity was 2.02 g/(l·h) for the first six cycles in an air-lift bioreactor [8]. Du et al. used two different physical forms (filamentous and pellet) to produce L-lactic acid by *R. oryzae* in semicontinuous fermentation; the volumetric productivities were 5.06 and 4.39 g/(l·h), respectively, in the second cycle, but decreased to 2.36 and 2.93 g/(l·h) in the third cycle [9]. Bai et al. carried out semicontinuous fermentation using *R. oryzae* R1021; the average volumetric productivity reached 3.51 g/(l·h) in the first six cycles, but the L-lactic acid concentration (LLAC) was only 64.5 g/l in the seventh cycle [5]. Liu et al. developed a novel process for coproduction of lactic acid and chitin using pelletized *R. oryzae* NRRL 395, in which for the first seven cycles the average LLAC and volumetric productivity were 66 g/l and 2.4 g/(l·h), respectively, but the volumetric productivity dropped to 1.2 g/(l·h) in cycle 9 [10]. In the above studies, volumetric productivity was improved by semicontinuous fermentation, but fewer than nine cycles were studied in detail. Although many researchers have tried to apply semicontinuous fermentations involving fungi pellets due to the high volumetric productivity and high utilization ratio of biomass, there are few reports concerning optimization of the feeding medium composition. Ions have to be of uniform concentration in the feeding medium to maintain the activities of key dehydrogenases and the growth of the fungi, and sufficient glucose has to be provided to obtain high production yield as well.

In this study, glucose was used as carbon source in the fermentation medium for L-lactic acid production by *R. oryzae* AS 3.819. The effects of inoculation size, $CaCO_3$ addition time, and temperature on L-lactic acid production and fungi morphology in the first cycle were studied. To maintain and improve the L-lactic acid yield, an orthogonal experimental design was used to determine the optimal feeding medium. Moreover, the activity of key lactate dehydrogenases (LDH) was investigated using semicontinuous fermentation in flask, and enhanced fermentation for L-lactic acid production was performed in a 7-l magnetically stirred fermentor.

Materials and methods

Microorganism

Rhizopus oryzae AS 3.819 purchased from China General Microbiological Culture Collection Center, conserved in the laboratory of the School of Biotechnology and Food Engineering, the Key Laboratory for Agricultural Products Processing of Anhui Province, Hefei University of Technology, was used in this study.

Culture medium

The preculture medium contained (g/l): glucose, 120; $(NH_4)_2SO_4$, 4; KH_2PO_4, 0.45; $ZnSO_4{\cdot}7H_2O$, 0.44; $MgSO_4{\cdot}7H_2O$, 0.25. The production medium contained (g/l): glucose, 120; $(NH_4)_2SO_4$, 4; KH_2PO_4, 0.14; NaH_2PO_4, 0.16; $ZnSO_4{\cdot}7H_2O$, 0.44; $MgSO_4{\cdot}7H_2O$, 0.25; $CaCO_3$ added at the beginning of the fermentation, 60. The feeding medium contained (g/l): glucose, 100; $(NH_4)_2SO_4$, 2; KH_2PO_4, 0.3; $ZnSO_4{\cdot}7H_2O$, 0.22; $MgSO_4{\cdot}7H_2O$, 0.25; $CaCO_3$, 50 [11].

Seed culture

The fungus was first grown on potato-dextrose agar (PDA) slant at 32°C. Three days later, fungal spores were collected with a platinum loop and suspended in sterilized water, i.e. spore suspension (concentration adjusted to 5×10^6 spores/ml). A 250-ml flask containing 50 ml preculture medium was sterilized at 121°C for 15 min, and each flask was inoculated with 5 ml spore suspension and incubated in a rotary shaker at 180 rpm at 32°C for 24 h, and this was seed culture.

Effects of inoculum size, $CaCO_3$ addition, and temperature on L-lactic acid production and *Rhizopus oryzae* AS 3.819 morphology in flask

Using monofactorial study method, morphology of *R. oryzae* AS 3.819 in flask was investigated. When changing one factor, the other factors were kept the same as follows: A 250-ml flask containing 50 ml production medium was inoculated 10% of seed culture and cultured at 32°C for 60 h with agitation speed of 200 rpm. To maintain pH 5.4–6.0 during culture, 3 g $CaCO_3$ was added to each flask.

Optimization of feeding medium for semicontinuous production of L-lactic acid in flask

The agitation and aeration were stopped until mycelial pellets precipitated to the bottom of the flask at the end of each cycle. Then the broth without pellets was poured out, and the sterilized feeding medium was added to the flask to begin the next cycle. The fermentation conditions were the same as those for the first cycle, except that the fermentation period was shortened to 24 h. Orthogonal experimental investigation was used to optimize the feeding medium composition. The experiments were carried out in triplicate.

L-lactic acid production and LDH activity using flask in semicontinuous fermentation

Semicontinuous fermentation was carried out in flask using the above optimal feeding medium. Each cycle lasted 24 h, and 2 g (wet weight) mycelia was collected to determine LDH activity at 12 h of each cycle.

L-lactic acid production using magnetically stirred fermentor in semicontinuous fermentation

A 7-l magnetically stirred fermentor with a vessel of 180 mm diameter and 7.0 dm^3 total volume was used in this study. The fermentor was equipped with a four-blade turbine stirrer. The blade was 64 mm in diameter and 16 mm in height. The center of the stirrer was 58 mm above the bottom of the vessel, and the bottom of the vessel was flat. An original basket with three baffles placed 5 mm off from the fermentor wall was used.

Culture was performed in working volume of 5 l at 32°C. The aeration rate was 1.0 l/(l·min), the inoculation was 4% of spore suspension, and agitation speed was 300 rpm. To prevent decrease in pH, 60 g/l sterilized $CaCO_3$ was added to the production medium.

After the first culture cycle, agitation and aeration were stopped until mycelial pellets precipitated to the bottom of the fermentor at the end of each cycle. Then 4.5 l broth was pumped out without pellets, and the sterilized feeding medium was added to the fermentor to begin the next cycle. The fermentation conditions were the same as for the first cycle, except for a 20 h fermentation period.

Analytical methods

L-lactic acid was extracted from fermented culture using 0.5 M H_2SO_4, diluted with distilled water, and filtered through a 0.22 μm membrane. LLAC was analyzed by high-performance liquid chromatography (HPLC) using a Purospher STAR C18 (Merck, USA) 250 × 4.6 (5 μm) column and ultraviolet (UV) detection at 210 nm. The eluent was 5 mM H_2SO_4 at flow rate of 0.8 ml/min.

Residual sugar concentration (RSC) was determined by dinitrosalicylic acid method [6]. Dry cell weight (DCW) was determined by washing the mycelia pellets twice with 4 M HCl to remove residual calcium carbonate, and the washed biomass was dried at 80°C for 24 h before weight analysis [6]. LDH activity was determined as described in the literature [12]. All values were measured in triplicate, with uncertainty within 5%.

Results and discussion

Effects of inoculum size, $CaCO_3$ addition, and temperature on L-lactic acid production and *R. oryzae* AS 3.819 morphology in flask culture

Inoculum size

The effects of inoculum size on L-lactic acid production and mycelial morphology in flask were studied in detail. Different concentrations of spore suspension and seed culture were inoculated into production medium, respectively. As shown in Table 1, uniform pellets were formed, and the LLAC was highest (93.33 g/l) with the highest DCW (3.85 g/l) when the inoculum size of spore suspension was 4%. More than 8% spore suspension led to flocculation of fungi mycelia, resulting in decrease of LLAC.

When seed culture was used for L-lactic acid production, as shown in Table 1, 10% seed culture was the optimal inoculum size, with the highest LLAC (93.20 g/l) but lower DCW than with 15% or 20% seed culture. It could be concluded that the highest DCW did not lead to the highest L-lactic acid yield; more nutrition was used for fungi growth, while a large inoculum size was inoculated, which led to a decrease of L-lactic acid yield. This conclusion agrees well with Martak et al. [4].

For flask culture, L-lactic acid yield was the same for 4% spore suspension or 10% seed culture. Only 200 ml spore suspension was needed to inoculate the 7-l bioreactor, considering the risk of contamination, so spore suspension provided suitable "seeds" for fermentation in the 7-l bioreactor. However, seed culture should be used in a scaled-up fermentation, because larger inoculum was needed and the lag time of fungi growth must be shortened. It should be noted that there were no pellets but rather small, short mycelia in the seed culture. However, in the research of Miura et al. [13], uniform pellets preformed in the seed culture. That study also noted that inoculum size had a significant effect on L-lactic acid yield, and especially on *R. oryzae* AS 3.819 morphology.

Table 1 Effects of time of $CaCO_3$ addition, inoculum, and temperature on L-lactic acid production and *Rhizopus oryzae* morphology in flask culture

		LLAC (g/l)	Yield (g/g)	DCW (g/l)	Morphology
Spore suspension (%)	2	82.00	0.68	2.24	Filamentous
	4	93.33	0.78	3.85	Pellets
	6	87.50	0.73	2.92	Pellets floc
	8	83.20	0.69	2.50	Small pellets floc
	10	69.30	0.58	2.12	Clumps floc
	12	60.10	0.50	1.30	Clumps
Seed culture (%)	2	45.30	0.38	1.58	Filamentous
	5	86.20	0.72	2.50	Filamentous, few pellets
	10	93.20	0.78	3.88	Pellets
	15	85.60	0.71	4.53	Mycelia floc
	20	76.50	0.64	4.12	Filamentous, few clumps
	30	59.60	0.50	2.12	Clumps
$CaCO_3$ addition time (h)	0	94.98	0.79	3.79	Pellets
	4	95.46	0.80	3.86	Pellets, few pellets floc
	8	93.56	0.78	3.92	Pellets floc
	12	88.30	0.74	3.69	Pellets floc, few clumps
	16	75.60	0.63	3.61	Clumps
	20	71.10	0.59	2.58	Clumps
	24	64.00	0.53	2.39	Clumps
Temperature (°C)	30	87.98	0.73	3.52	Filamentous, few pellets
	32	95.86	0.80	3.89	Pellets
	34	96.69	0.81	4.15	Pellets, few filamentous
	36	84.10	0.70	4.65	Clumps, few filamentous
	38	72.00	0.60	3.95	Clumps

$CaCO_3$ addition time

L-lactic acid production and *R. oryzae* AS 3.819 mycelial morphology for different $CaCO_3$ addition times are presented in Table 1. The results show that L-lactic acid production decreased from 93.56 to 64.00 g/l when the time of $CaCO_3$ addition was more than 12 h after inoculation. Fungi biomass increased quickly with a dramatic growth rate from 12 to 24 h, during which period most of the mycelia were filaments, which could embed $CaCO_3$ and form clumps. These clumps blocked nutrient transfer, resulting in a decrease in production efficiency in the broth. On the contrary, it was reported by Bai et al. that the optimal $CaCO_3$ addition time was 8 h after culture and that clumps formed when $CaCO_3$ was added before 8 h [5]. As shown in Table 1, when $CaCO_3$ was added before 8 h, most of the mycelia were in pellet form, which was beneficial for high L-lactic acid yield.

Temperature

There are few published studies on the effects of temperature on growth and metabolites for filamentous fungi. Liu et al. determined the optimal temperature for L-lactic acid production by *R. oryzae* NRRL395 [14]. Although temperature is an environmental parameter that is easy to control, changes in temperature produce simultaneous changes in other culture variables. An increase in incubation temperature within physiological ranges enhances the growth rate. Also, the temperature affects fungi morphology [15]. In this work the optimal temperature for L-lactic acid production and pellet formation by *R. oryzae* AS 3.819 was investigated. As $CaCO_3$ is the neutralizer in L-lactic acid production, calcium lactate is formed in the broth, the solubility of which increases with increasing temperature. The L-lactic acid yield decreased from 96.69 to 72.00 g/l with increasing temperature from 34°C to 38°C. However, the L-lactic acid yield increased from 87.98 to 96.69 g/l with increasing temperature from 30°C to 34°C, as shown in Table 1. At temperatures above 34°C, the fungi flocculated together, wrapping $CaCO_3$, to form clumps, leading to a decrease in L-lactic acid yield. The optimal temperature for high L-lactic acid yield and uniform pellets was 32–34°C.

Optimization of feeding medium for semicontinuous production of L-lactic acid

The standard $L_{16}(4^5)$ matrix and statistical data on the factors were computed in detail, based on literature methods [16]. Tables 2 and 3 show the standard orthogonal matrix,

Table 2 Standard $L_{16}(4^5)$ matrix and L-lactic acid yield

	Glucose (g/l)	$(NH_4)_2SO_4$ (g/l)	KH_2PO_4 (g/l)	$ZnSO_4{\cdot}7H_2O$ (g/l)	$MgSO_4{\cdot}7H_2O$ (g/l)	L-lactic acid yield (%)				
						1	2	3	Average	Standard deviation
1	1 (70)	1 (1)	1 (0.005)	1 (0.11)	1 (0.15)	75.62	76.16	75.95	75.91	0.272
2	1	2 (2)	2 (0.075)	2 (0.22)	2 (0.25)	81.23	80.23	78.63	80.03	1.311
3	1	3 (3)	3 (0.1)	3 (0.33)	3 (0.35)	80.25	79.65	79.1	79.67	0.575
4	1	4 (4)	4 (0.125)	4 (0.44)	4 (0.45)	65.23	63.25	65.01	64.5	1.085
5	2 (80)	1	2	3	4	73.89	74.29	74.16	74.11	0.204
6	2	2	1	4	3	69.89	71.36	68.39	69.88	1.485
7	2	3	4	1	2	77.68	78.95	78.56	78.4	0.651
8	2	4	3	2	1	73.12	75.09	73.95	74.05	0.989
9	3 (90)	1	3	4	2	67.58	68.54	68.19	68.1	0.486
10	3	2	4	3	1	78.16	80.19	78.01	78.79	1.218
11	3	3	1	2	4	73.15	73.16	73.95	73.42	0.459
12	3	4	2	1	3	69.89	71.09	70.23	70.4	0.619
13	4 (100)	1	4	2	3	75.36	76.12	76.12	75.87	0.439
14	4	2	3	1	4	80.53	78.96	79.64	79.71	0.787
15	4	3	2	4	1	75.85	75.65	74.19	75.23	0.906
16	4	4	1	3	2	78.31	77.13	76.3	77.25	1.010
k_1	900.31	881.98	889.37	913.26	911.94	$C = 267895.118$				
k_2	889.33	925.22	899.33	910.11	911.33					
k_3	872.14	920.14	904.6	929.44	887.45					
k_4	924.16	858.6	892.64	833.13	875.22					
SS	118.7	252.58	11.61	463.91	82.76					

Table 3 Statistical data on the factors

Factor	SS	Degrees of freedom	*F* ratios
Glucose (g/l)	118.7	3	53.12
$(NH_4)_2SO_4$ (g/l)	252.58	3	113.04
KH_2PO_4 (g/l)	11.61	3	5.2
$ZnSO_4{\cdot}7H_2O$ (g/l)	463.91	3	207.62
$MgSO_4{\cdot}7H_2O$ (g/l)	82.76	3	37.04
Error	23.834	32	
Total	953.394	47	

sum of the response, sum of squares (SS), and *k* values of the factors.

From Table 3, it can be concluded that all the factors [glucose, $(NH_4)_2SO_4$, KH_2PO_4, $ZnSO_4{\cdot}7H_2O$, $MgSO_4{\cdot}7H_2O$] had significant influence on L-lactic acid yield at the 95% confidence level, with *F*-ratios of 53.12, 113.04, 5.2, 207.62, and 37.04, respectively. The results also showed the optimum composition to be (g/l): glucose 100, $(NH_4)_2SO_4$ 2, KH_2PO_4 0.1, $ZnSO_4{\cdot}7H_2O$ 0.33, $MgSO_4{\cdot}7H_2O$ 0.15, $CaCO_3$ 50.

In the semicontinuous fermentation process, as the pellet biomass was stably held at certain values, the duration of fungal growth was notably shortened. However, the fermentation time in the following cycle was shortened. Semicontinuous fermentation with *R. oryzae* pellets was an effective method to improve L-lactic acid volumetric productivity.

L-lactic acid production using flask in semicontinuous fermentation

Figure 1 shows that 20 cycles of semicontinuous fermentation were successfully carried out with flask culture. The final LLAC of the first cycle reached 94.5 g/l, L-lactic acid yield was 78.75%, and volumetric productivity was 1.58 g/(l·h). In repeated cycle fermentation, the final L-lactic acid concentration reached 64–72 g/l, L-lactic acid yields were 80–90%, and fermentation was completed in 24 h for each cycle.

It is known that volumetric productivity of L-lactic acid by fungi relies on lactate dehydrogenase (LDH) activity [12]. In Fig. 1, no significant changes were observed in LDH activity in 19 repeated fermentation cycles, except for the first cycle fermentation. The reason may be that, with

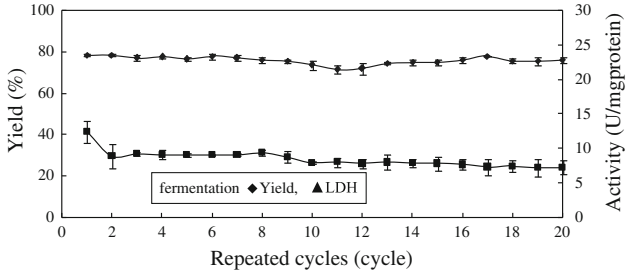

Fig. 1 Stability of L-lactic acid yield and LDH activity by *Rhizopus oryzae* in semicontinuous fermentation: yield (*filled diamonds*) and LDH (*filled triangles*)

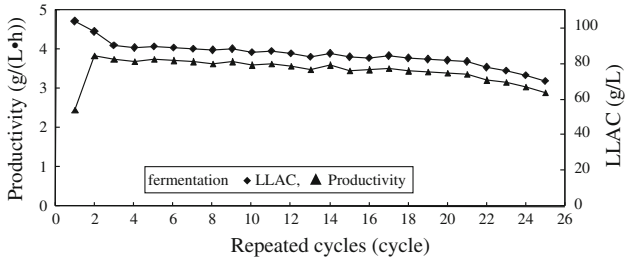

Fig. 2 Stability of L-lactic acid production and productivity by *Rhizopus oryzae* in semicontinuous fermentation: LLAC (*filled diamonds*) and productivity (*filled triangles*)

the addition of fresh medium, LDH was activated again in each cycle. Moreover, the LDH activity ranged from 7.2 to 9.2 U/mg in 19 cycles of repeated fermentation, which was why the L-lactic acid yield stayed above 80% in the repeated fermentation cycles.

L-lactic acid production using magnetically stirred fermentor in semicontinuous fermentation

Semicontinuous fermentation for 25 cycles using pellets of *R. oryzae* was performed in a 7-l magnetically stirred fermentor at the optimal values obtained from flask culture in Fig. 2. The final LLAC of the first cycle reached 103.7 g/l, and volumetric productivity was 2.16 g/(l·h). In the following 19 cycles of repeated fermentation, the final LLAC reached 81–95 g/l and volumetric productivities were 3.40–3.85 g/(l·h), those values being higher than those reported by Yin et al. [8] and Bai et al. [5]. In the last five cycles (cycles 21–25), because of pellet rupture and fungi autolysis, the metabolic capability of *R. oryzae* decreased, leading to decrease of volumetric productivity and LLAC, from 3.37 to 2.91 g/(l·h) and from 80 to 71 g/l, respectively.

In semicontinuous fermentation with *R. oryzae* pellets, most pellets were retained well, being about 1.0–2.5 mm in size, as shown in Fig. 3. At the end of the first cycle, most of the mycelial were uniform pellets, being about 0.5–1.2 mm in size, and there were fewer clumps. The number of clumps increased as the semicontinuous fermentation

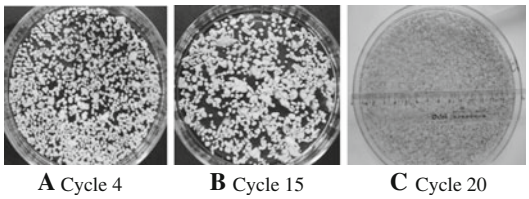

Fig. 3 *Rhizopus oryzae* morphology in semicontinuous fermentation with fermentor: **a** pellets with 1.0–2.0 mm diameter in cycle 4, **b** pellets with 1.5–2.5 mm diameter in cycle 15, and **c** pellets in cycle 20

continued, with pellet size increasing to 1.0–2.0 mm in cycle 4, 1.5–2.5 mm in cycle 15, and 2.0–3.0 mm in cycle 20. Fungi were growing into new biomass, while old pellets were breaking and autolysing during the semicontinuous fermentation. As a result, the volumetric productivity changed little (Fig. 2). The results demonstrate that L-lactic acid production was due to combined work by old pellets and the new biomass.

Conclusions

Pelletized morphology significantly enhanced L-lactic acid production. This study used a monofactorial study method to develop a culture method to form uniform pellets of *R. oryzae* AS 3.819, and optimized the feeding medium for semicontinuous fermentation, resulting in many more repeated cycles with high volumetric productivity. LDH activity during semicontinuous fermentation was studied preliminarily. Semicontinuous fermentation was successfully conducted in a 7-l magnetically stirred fermentor. It was evident that semicontinuous fermentation was a good method to improve L-lactic acid volumetric productivity by *R. oryzae* AS 3.819. Further studies on fungi growth characteristics and metabolic processes during semicontinuous fermentation are needed.

Acknowledgments Financial support from Hi-tech Research and Development Program of China (863 Program) and Program of the Key Laboratory for Agricultural Products Processing of Anhui Province (2009AKSY0102) are gratefully acknowledged.

References

1. Jin QR, Wang BY (2000) Production and application handbook for organic acid from fermentation. Chin L Ind, Beijing
2. Rojan PJ, Nampoothiri KM, Pandey A (2007) Fermentative production of lactic acid from biomass: an overview on process developments and future perspectives. Appl Microbiol Biotechnol 74:524–534
3. Zhang ZY, Jin B, Kelly JM (2007) Production of lactic acid from renewable materials by *Rhizopus* fungi. Biochem Eng J 35:251–263
4. Martak J, Schlosser S, Sabolova E, Kristofikova L, Rosenberg M (2003) Fermentation of lactic acid with *Rhizopus arrhizus* in a

stirred tank reactor with a periodical bleed and feed operation. Process Biochem 38:1573–1583
5. Bai DM, Jia MZ, Zhao XM, Ban R, Shen F, Li XG, Xu SM (2003) L(+)-lactic acid production by pellet-form *Rhizopus oryzae* R1021 in a stirred tank fermentor. Chem Eng Sci 58:785–791
6. Liao W, Liu Y, Frear C, Chen SL (2007) A new approach of pellet formation of a filamentous fungus—*Rhizopus oryzae*. Bioresource Technol 98:3415–3423
7. Yu MC, Wang RC, Wang CY, Duan KJ, Sheu DC (2007) Enhanced production of L(+)-lactic acid by floc-form culture of *Rhizopus oryzae*. J Chin Inst Chem Eng 38:223–228
8. Yin PM, Yahiro K, Ishigaki T, Park Y, Okabe M (1998) L(+)-lactic acid production by repeated batch culture of *Rhizopus oryzae* in air-lift bioreactor. J Ferment Bioeng 85:96–100
9. Du JX, Cao NJ, Gong CS, Tsao GT (1998) Production of L-lactic acid by Rhizopus oryzae in a bubble column fermentor. Appl Biochem Biotechnol 70–72:323–329
10. Liu Y, Liao W, Chen S (2008) Co-production of lactic acid and chitin using a pelletized filamentous fungus *Rhizopus oryzae* cultured on cull potatoes and glucose. J Appl Microbiol 105:1521–1528
11. Zhu Y (2006) Production of L-lactic acid by self-immobilized *Rhizopus oryzae*. Dissertation Hefei University of Technology, Hefei
12. Buyukkileci a O, Hamamci H, Yucel M (2006) Lactate and ethanol productions by *Rhizopus oryzae* ATCC 9363 and activities of related pyruvate branch point enzymes. J Biosci Bioeng 102:464–466
13. Miura S, Arimura T, Hoshino M, Kojima M, Dwiarti L, Okabe M (2003) Optimization and scale-up of L-lactic acid fermentation by mutant strain Rhizopus sp MK-96–1196 in airlift bioreactors. J Biosci Bioeng 96:65–69
14. Liu Y, Liao W, Liu CB, Chen SL (2006) Optimization of L-(+)-lactic acid production using pelletized filamentous *Rhizopus oryzae* NRRL 395. Appl Biochem Biotechol 131:844–853
15. Papagianni M (2004) Fungal morphology and metabolite production in submerged mycelial processes. Biotechnol Adv 22:189–259
16. Oke IA (2009) Orthogonal experiments in the development of carbon-resin for chloride removal from solutions. Stat Methodol 6:109–119