J Ind Microbiol Biotechnol (2012) 39:1377–1384
DOI 10.1007/s10295-012-1138-5

Enhanced production of poly(3-hydroxybutyrate) in a novel airlift reactor with in situ cell retention using *Azohydromonas australica*

Geeta Gahlawat · Bedoshree Sengupta · Ashok K. Srivastava

Received: 2 January 2012 / Accepted: 18 April 2012 / Published online: 4 July 2012

Abstract Economic production of biodegradable plastics is a challenge particularly because of high substrate and energy cost inputs for its production. Research efforts are being directed towards innovations to minimize both of the above costs to economize polyhydroxybutyrate (PHB) production. A novel airlift reactor (ALR) with outer aeration and internal settling was utilized in this investigation. Although it featured no power consumption for agitation, it facilitated increased oxygen transfer rate and better cell retention than stirred tank reactor (STR), thereby resulting in enhanced PHB productivity. ALR with in situ cell retention demonstrated a significant improvement in biomass concentration and biopolymer accumulation. The total PHB production rate, specific biomass, and product yield in the ALR were observed to be 0.84 g/h, 0.43 g/g, and 0.32 g/g, respectively. The studies revealed that the volumetric oxygen mass transfer rate and mixing time for ALR were 0.016 s^{-1} and 3.73 s, respectively, at 2.0 vvm as compared with corresponding values of 0.005 s^{-1} and 4.95 s, respectively, in STR. This demonstrated that ALR has better oxygen mass transfer and mixing efficiency than STR. Hence, ALR with cell retention would serve as a better bioreactor design for economic biopolymer production than STR, particularly due to its lower cost of operation and simplicity along with its enhanced oxygen and heat transfer rates.

G. Gahlawat · A. K. Srivastava (✉)
Department of Biochemical Engineering and Biotechnology, Indian Institute of Technology Delhi, Hauz Khas 110016, New Delhi, India
e-mail: ashokiitd@hotmail.com; ashokks@dbeb.iitd.ac.in

B. Sengupta
Manipal Institute of Technology, Manipal 576104, Karnataka, India

Keywords Polyhydroxybutyrate · *Azohydromonas australica* · Airlift reactor · Mass transfer · Mixing time

Introduction

Poly(3-hydroxybutyrate) (PHB) is an interesting biodegradable and biocompatible thermoplastic which is usually produced by various microorganisms intracellularly as energy reserve material in response to limiting nutrient conditions of nitrogen, phosphorus, and magnesium [2]. It has properties very similar to commercially used petrochemically derived thermoplastic in terms of molecular weight, brittleness, stiffness, melting point, and glass-transition temperature [3, 17]. PHB can be produced by microorganisms from renewable resources such as glycerol, molasses, soya milk wastes, and plant oils under normal temperature and pressure conditions, unlike the petrochemically derived plastics that are produced by fractionation of depleting fossil fuels with consumption of huge amounts of energy [18]. Hence, enormous research efforts are being directed in this area in order to use PHB as a replacement for conventional petrochemically derived plastics.

In the present study *Azohydromonas australica* (earlier known as *Alcaligenes latus* DSM 1124) was selected for biopolymer production because it produces PHB particularly during the growth phase of cultivation. Moreover, *A. australica* can utilize various inexpensive substrates such as sucrose, beet molasses [24], soya and malt wastes [27], and maple sap [26] for production of PHB. The main aim of the present study was to investigate economic production of PHB by enhancement of its concentration, yield, and productivity from culture which not only features growth on inexpensive substrates but also accumulates PHB during the entire log phase; therefore, *A. australica* was selected for

this purpose. It was observed that it can accumulate PHB up to 80 % of dry cell weight when grown on inexpensive substrates such as sucrose [6], which will further reduce recovery and equipment-related costs [9]. Further minimization of production cost in the present investigation was attempted by using a novel airlift reactor (ALR) with outer aeration and internal settling configuration which featured cultivation without agitation (or the associated power consumption) yet facilitated increased oxygen transfer rate with improved cell settling (due to in situ cell retention).

ALRs are a special class of bubble column reactors (pneumatic reactors) having net-draft tube without any mechanical components such as agitators and seals [23]. The mechanical simplicity of the ALR, with no rotating shaft or impellers, results in reduction of construction and operation costs as well as less risks of contamination. In this study a relatively new design of ALR with a special arrangement of cell settling was used as shown in Fig. 1a, particularly because of the numerous advantages it provides: a definite fluid flow pattern resulting in high oxygen and heat transfer [7], lower mixing time, better cell retention resulting in higher cell density, less power input due to the absence of mechanical agitation, simple construction, and good aseptic control [4].

It has been reported in literature that cultivation studies carried out in an ALR are technically and economically optimal for production of various substances as compared with use of stirred tank reactor (STR) [11, 22]; for instance, in a comparison of STR and ALR for production of polygalacturonases by *Aspergillus oryzae*, it was concluded that ALR had the potential for use in large-scale production because of its lower operation costs, performance similar to STR, and better oxygen transfer rates [11]. In another study, Saravanan et al. [22] showed that *Pseudomonas* spp. could degrade phenol in 47 h in an internal loop ALR while an STR operating in batch mode took 8 days to degrade phenol completely. Thus, the performance of internal loop ALR in terms of phenol degradation was also better than that of batch STR. Because of the economical feasibility of ALR, these reactors have also been used for production of other compounds also, such as lovastatin from *Aspergillus terreus* [13] and ligninolytic enzymes from *Phanerochaete chrysosporium* [10].

In the present investigation, ALR with an in situ cell retention device was used for growth-associated production of PHB by *A. australica* and a comparative study with STR was done to ascertain its suitability for economic PHB production in terms of concentration, yield, and productivity. To the best of our knowledge, there are no reports in literature on growth-associated production of PHB by *A. australica* by airlift reactor (outer aeration and internal settling configuration) with in situ cell retention.

Materials and methods

Microorganism and medium composition

A. australica DSM 1124 obtained from the German Collection of Microorganisms and Cell Cultures (DSMZ, Germany) was used in the present study. The strain was

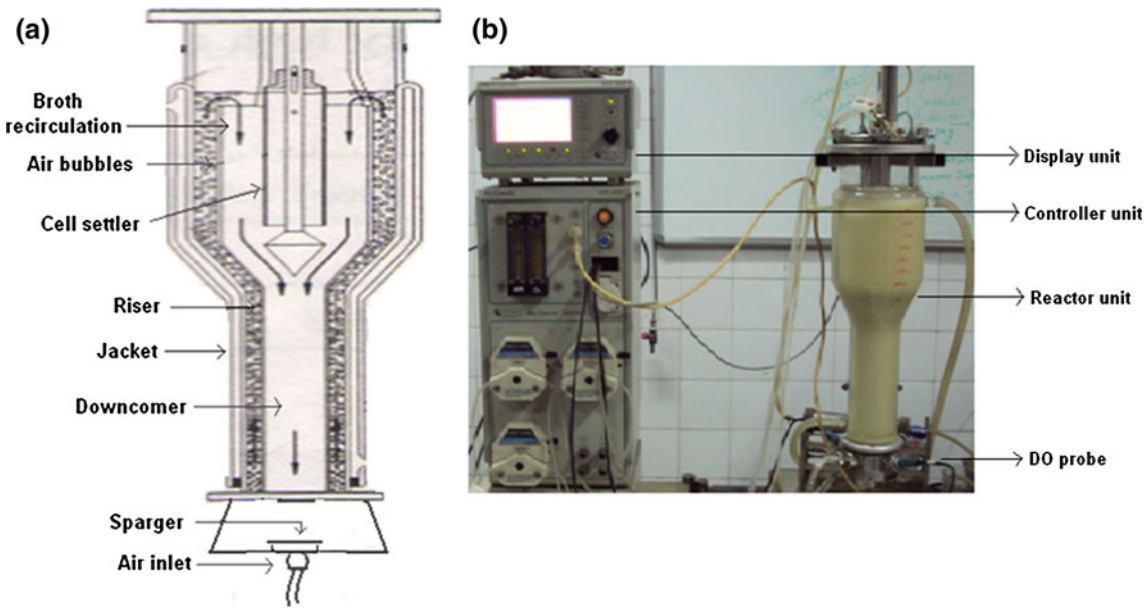

Fig. 1 **a** Schematic representation and **b** experimental setup of an airlift reactor with outer aeration and internal settling (OAIS); Applikon Dependable Instrument, The Netherlands

maintained on nutrient agar (HiMedia, India) slants at 4 °C and subcultured monthly onto new agar slants.

After initial screening of medium components by Plackett–Burman protocol [20], response surface methodology (RSM) was used to determine the optimized medium recipe for cell growth and PHB accumulation. The statistical medium design was facilitated by Design Expert (version 5.0.9) software (Stat-Ease Corporation, USA). The composition of the statistically optimized medium recipe was as follows: sucrose, 25 g/L; NH_4OH, 2.8 g/L; $MgSO_4$, 0.2 g/L; KH_2PO_4, 3.25 g/L; Na_2HPO_4, 3.25 g/L; trace element solution (TES), 1.5 mL/L. TES consisted of: 6 g/L ammonium Fe(III) citrate, 10 g/L $CaCl_2{\cdot}2H_2O$, 0.3 g/L H_3BO_3, 0.2 g/L $CoCl_2{\cdot}6H_2O$, 0.1 g/L $ZnSO_4{\cdot}7H_2O$, 0.03 g/L $MnCl_2{\cdot}4H_2O$, 0.03 g/L $Na_2MoO_4{\cdot}2H_2O$, 0.02 g/L $NiSO_4{\cdot}7H_2O$, and 0.01 g/L $CuSO_4{\cdot}5H_2O$. All medium components except TES were autoclaved at 121 °C (15 psi) for 20 min and then cooled to 33 °C. For preparation of medium, the phosphates were autoclaved separately to prevent precipitation and TES was filter-sterilized. All components were then mixed aseptically under laminar flow. The pH of the medium was adjusted aseptically to 7.0 using 2 N NaOH/HCl.

Inoculum development for the reactor

Two loopfuls of culture from the agar slants were inoculated aseptically into 250-mL flasks containing 50 mL statistically optimized medium (described in Sect. 2.1). The flasks were then kept on a rotary shaker at 200 rpm and 33 °C for 48 h. After cells had grown considerably for 2 days and reached exponential phase (as predetermined by shake flask studies), the culture was used as inoculum. Before reactor operation, the culture was further inoculated (5 % v/v) into 500-mL flasks containing 100 mL optimized medium and allowed to grow for 24 h till the log phase was obtained. The culture so obtained was used to inoculate the ALR and STR.

Reactor configuration and experimental setup

Airlift reactor (4 L total capacity) having outer aeration and internal settling configuration (Applikon Dependable Instruments, The Netherlands) with working volume of 2.5 L equipped with pH, temperature, and dissolved oxygen (DO) probes was used, as shown in Fig. 1b. The innovative feature of this reactor is air bubble disengagement in the upper portion of the reactor and the associated cell settling as the liquid travel backs to the bottom of the reactor for recirculation, as shown in Fig. 1a. The temperature was maintained at 33 °C by circulating constant-temperature (33 °C) water in the cooling jacket from a chilled water circulator (Julabo FP50, Germany). Air was sparged from the bottom of the reactor using a wire mesh stainless-steel sparger. The air flow rate was set at 2.0 vvm initially. The DO concentration was maintained above 30 % saturation value by manually adjusting the flow rate of sterile air. The culture pH was maintained at 7.0 by automatic addition of 2 N NaOH/2 N HCl through a pH controller unit. The DO concentration in the bioreactor was measured using an in situ (Applisens) DO probe installed laterally near the bottom end of the bioreactor (Fig. 1b).

For comparison, a 7-L stirred tank bioreactor (STR) (Applikon Dependable Instruments, The Netherlands) with working capacity of 4 L was used under the same cultivation conditions as mentioned above. The reactor was equipped with two conventional flat-blade turbine-type impellers and three baffles. Air was sparged from the bottom of a perforated L-shaped tube having very small holes of 0.2 mm size. The holes in the sparger were located at the bottom of the tube to ensure that the medium was driven out by the gas stream. Initially, the air flow rate and rotation speed of the impeller were set at 2.0 vvm and 200 rpm, respectively.

The batch cultivation experiments in both reactors were done in triplicate to confirm the reproducibility of the results, and samples were withdrawn at regular intervals of 3 h from the sampling port to estimate biomass, PHB, residual sucrose, and nitrogen concentrations. Both reactors were operated till 48 h to get an idea about the entire cultivation kinetics, particularly the end of log phase and start of stationary phase. However, it was observed from the batch kinetics that maximum biomass and PHB concentrations in the STR and ALR configurations were achieved at 36 and 39 h, respectively. Therefore, fermentation in STR and ALR can be considered to be over at these respective time intervals, and cells can be harvested from the reactor.

Analytical methods

Estimation of biomass, PHB, residual sucrose, and nitrogen

The optical density of the samples was measured at 600 nm against a medium blank using a spectrophotometer (OPTIZEN model 3220UV, Mecasys, Korea). Biomass was determined by a standard plot between OD_{600nm} versus dry cell weight (DCW). Fermentation samples were collected at time intervals of 3 h and centrifuged at 10,000 rpm for 15 min at 4 °C. The supernatant obtained by centrifugation of the culture broth was used for residual substrate analysis. Residual sucrose concentration in suitably diluted supernatant was determined by the dinitrosalicylic acid (DNS) method [19]. The total nitrogen assay was done by measuring the residual ammonia by Kjeldahl method [15].

The cell pellet was dried at 90 °C in a hot air oven till constant weight was obtained, and DCW was calculated. After that, the cell pellet was used for PHB analysis by gas chromatography (GC 2010 Shimadzu Co., Japan) using benzoic acid as internal standard [21]. Each sample was injected three times for analysis, and the quantity of PHB was calculated from averaged values in this study.

Determination of initial volumetric oxygen transfer coefficient (K_La) and mixing time (t_m)

The volumetric oxygen transfer coefficient (K_La) was determined by dynamic gassing-out method with agitation and aeration rate set at 200 rpm and 2.0 vvm, respectively [25]. Mixing time was estimated by decolorization method using phenolphthalein solution as color indicator [5].

Estimation of kinetic parameters

The biomass ($Y_{X/S}$) and PHB yield ($Y_{P/S}$) from sucrose and PHB volumetric productivity (P) were defined as follows:

$$Y_{P/S} = \frac{\Delta P}{\Delta S}, \quad Y_{X/S} = \frac{\Delta X}{\Delta S}$$

$$P = \frac{\Delta P}{t_f - t_i},$$

where ΔP and ΔX are the concentration of PHB and biomass, respectively, produced in time interval $t_{(final)} - t_{(initial)}$, and ΔS is the concentration of sucrose consumed in the same time interval.

The total PHB content (P/X) was also calculated, where X and P are the maximum biomass and PHB concentration, respectively. The maximum specific growth rate (μ_{max}) was calculated from the slope of ln X versus time data during the exponential phase of culture.

Results and discussion

Bioreactor cultivation and kinetics profile study

Batch growth and product kinetics of *A. australica* were studied in ALR (with in situ cell retention) and STR under controlled environmental conditions to demonstrate the performance of both reactors with respect to biomass and PHB accumulation. All batch reactor experiments were conducted using the statistically optimized medium recipe (described above). Figures 2 and 3 show the time course of biomass, PHB accumulation, and substrate consumption during batch cultivation in ALR and STR, respectively. Experimental data points in Figs. 2 and 3 reflect average values of biomass, PHB, and residual nutrients at different time intervals. In both cases, a negligible lag phase (3 h) was observed, which can be attributed to the fact that the reactor was inoculated with actively growing exponential-phase cells. It was observed (Figs. 2, 3) that synthesis of PHB started immediately after the lag phase of 3 h, because PHB accumulation is growth-associated in *A. australica* [14], and no specific nutrient and/or oxygen limitation was necessary to trigger biopolymer accumulation as is usually the case in cultivation of *Ralstonia eutropha* [23]. Even in the present study sucrose was used as the carbon source, being much less expensive than glucose and fructose, for cost-effective production of PHB without nutrient limitation [9].

The ALR cultivation featured accumulation of enhanced biomass and PHB concentration of 10.76 and 7.81 g/L, respectively, with practically no unconverted substrates at the end of fermentation. On the other hand, cultivation in STR yielded 8.31 g/L biomass and 5.45 g/L PHB concentration in 36 h (Fig. 3), much lower than the corresponding values in ALR. ALR featured a total of 30 and 43 % enhancement in biomass and PHB concentration, respectively, as compared with STR. The total biomass and PHB concentration inside the ALR at time of harvesting were observed to be 43.05 and 31.24 g, respectively, as compared with corresponding values of 33.24 and 21.8 g, respectively, in STR. The high biomass and PHB accumulation in ALR as compared with STR were due to enhanced oxygen transfer capability, better mixing, and the cell settling property of the bioreactor [8]. In ALR, continued growth of the culture and PHB accumulation were observed till 39 h, particularly due to the availability of oxygen throughout the fermentation. The PHB concentration started to decline from 36 h in STR because of the

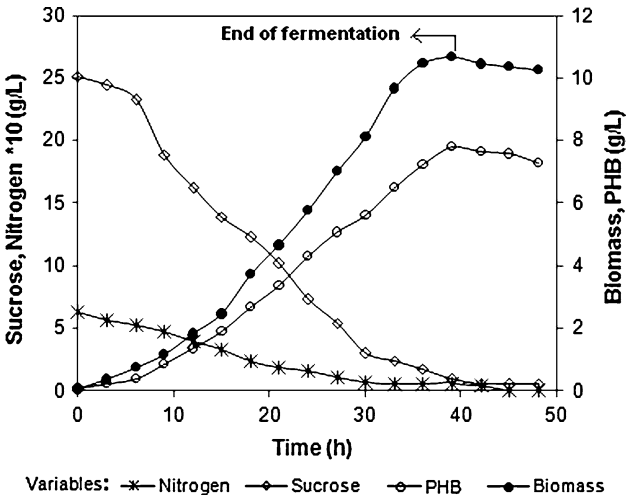

Fig. 2 Time course of biomass, PHB, and residual nutrients during batch cultivation in airlift reactor; process variables (biomass, PHB, sucrose, and nitrogen) show average values of three identical batch experiments

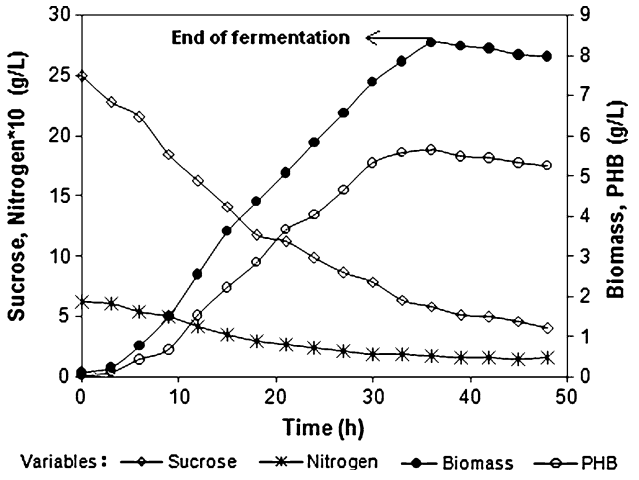

Fig. 3 Batch growth and product kinetics of *Azohydromonas australica* in stirred tank reactor; process variables (biomass, PHB, sucrose, and nitrogen) reflect average values of three identical batch experiments

onset of stationary phase. In ALR, the stationary phase was reached after 39 h of cultivation; therefore, culture showed greatly prolonged exponential phase. It was observed from the batch kinetics (Fig. 2) that the concentration of key limiting nutrients (sucrose and nitrogen) decreased significantly (primarily reaching a very low level) at 40 h; thus, major nutrients were essentially not available to support growth of the microorganism, with the result that the rates and specific rates of cell biomass formation started to decrease as well. Since PHB accumulation is growth-associated in case of *A. australica*, its production also stopped. At this point of time, cultivation could be converted to fed-batch (or semicontinuous), where fresh feeding of nutrients to the bioreactor will ensure availability of nonlimiting concentrations of the major nutrients. This scenario of cultivation could further improve the growth of the culture and product formation, which may eventually lead to high yield and productivity of PHB in the bioreactor.

Estimation of aeration and kinetics parameters

The kinetics analysis showed that sucrose and nitrogen were completely consumed in case of ALR, resulting in higher cell density and more PHB accumulation, with overall PHB productivity of 0.21 g/L h, which was higher than for STR (0.14 g/L h). During the entire cultivation period, 24.73 g/L sucrose was consumed out of an initial value of 25 g/L and only 0.27 g/L remained at the end of fermentation in ALR (Fig. 2), demonstrating that no oxygen limitation was experienced by the culture. In case of STR, approximately 21 g/L sucrose concentration was metabolized from an initial value of 25 and 4–5 g/L sucrose along with 0.175 g/L nitrogen was left unconverted in the fermentation broth at the end of cultivation (Fig. 3), which can be explained by the abrupt arrest of metabolic activities of the culture due to severe oxygen limitation towards the end of fermentation (Fig. 4a). The DO could not be increased further even by increasing the air flow rate in the bioreactor in the present study. This may be primarily due to the STR reactor geometry, for which it was not possible to increase the aeration efficiency beyond a certain value. Moreover, further increase of the aeration rate would give rise to foaming inside the bioreactor, thereby resulting in contamination problems. Thus, oxygen availability or aeration throughout the fermentation (achievable with the present configuration of ALR as shown in Fig. 4b) turned out to be an important requirement for attaining the maximum cell concentration with high PHB content in case of *A. australica*. Figure 4a and b demonstrate the DO concentration profiles of STR and ALR, respectively.

In case of ALR, the high PHB content of 73 % of DCW also demonstrates the economic feasibility of ALR with in situ cell retention for PHB production using *A. australica*. On the other hand, in STR, the PHB content was

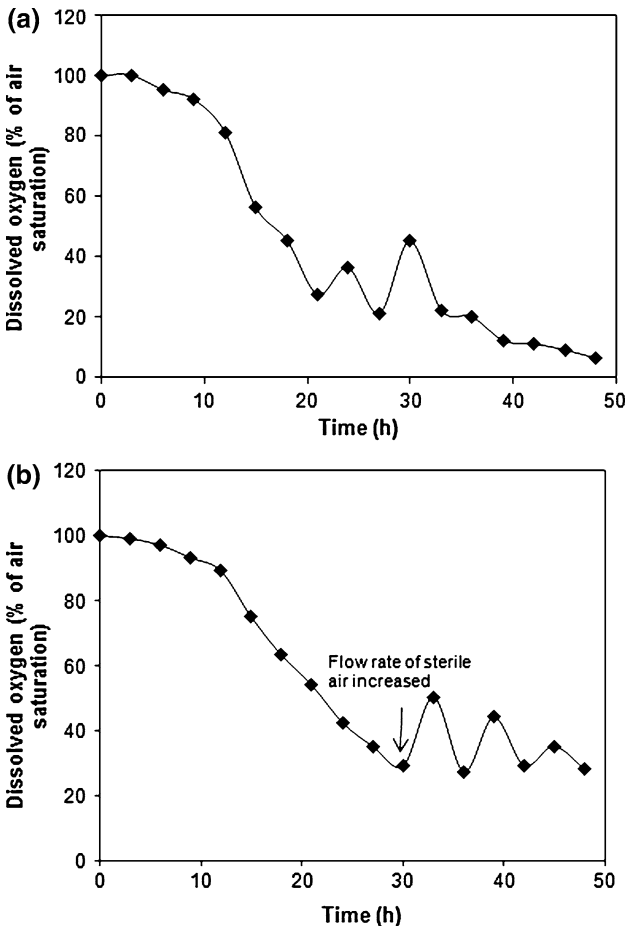

Fig. 4 Dissolved oxygen concentrations profile in **a** stirred tank reactor and **b** airlift reactor

Table 1 Comparison of key kinetics parameters of airlift reactor with stirred tank reactor in optimized medium

Reactor configuration	μ_{max}* (h^{-1})	Maximum biomass (g/L)	Maximum PHB (g/L)	$Y^{a}_{X/S}$(g/g)	$Y^{b}_{P/S}$(g/g)	PHB production rate (g/h)
STR batch cultivation	0.38	8.31	5.45	0.36	0.25	0.61
ALR batch cultivation	0.45	10.76	7.81	0.43	0.32	0.84

STR stirred tank reactor, *ALR* airlift reactor; cultivation time: 48 h (in both reactors)

* Maximum specific growth rate

[a] $Y_{X/S}$ is biomass yield coefficient based on sucrose

[b] $Y_{P/S}$ is product yield coefficient based on sucrose

calculated to be 65 % of DCW. It was also noted that the high PHB content in the case of ALR further improved the efficiency of the PHB recovery process and PHB yield (based on the carbon source), as also reported by Choi and Lee [9]. The maximum specific growth (μ_{max}) rate of *A. australica* during log phase was found to be 0.45 h^{-1} in ALR, suggesting that the culture grew at faster rates as compared with in STR ($\mu_{max} = 0.38\ h^{-1}$). The maximum PHB production rate, specific biomass yield ($Y_{X/S}$), and specific product yield ($Y_{P/S}$) on sucrose were observed to be 0.84 g/h, 0.43 g/g, and 0.32 g/g, respectively, in case of ALR, representing a significant improvement as compared with the corresponding values of STR presented in Table 1. On the basis of the batch kinetics and kinetic parameter data in this investigation, it was concluded that ALR performance in terms of growth-associated PHB production is better than that of STR.

The ALR used in the experiments was unique, as it showed bubble disengagement at the top and bubble-free fermentation broth featuring cell settling while circulating back to the bottom of the reactor (Fig. 1a), resulting in higher cell density that ultimately led to more PHB accumulation. This unique phenomenon of cell settling and bubble disengagement at the top of the reactor was the key factor leading to high productivity (0.21 g/L h) and yield (0.32 g/g) in the ALR. Gavrilescu and Tudose [12] have also shown that the geometry of the system in case of ALR must be carefully selected, since it strongly affects the mixing time and volumetric oxygen transfer coefficient. In the present configuration of ALR, a stream of air (bubbles) was fed from the bottom of the reactor, flowing along with the medium upwards through the riser. Once it reached the top of the reactor, the liquid velocity decreased, leading to bubble disengagement due to widening of the upper portion of the reactor. The liquid with significantly less air bubbles was channeled down through the downcomer with the help of the cell settler, resulting in higher cell concentration. Therefore, the DO concentration in the downcomer section shown in Fig. 1a would ideally be low in comparison with in the riser section. Since the DO probe was installed at the bottom of the bioreactor vessel, the DO concentration could be measured only in the region of liquid at the bottom of the reactor. Under rapid circulation of the medium in the reactor, it can be assumed that the DO concentration measured by the probe is the equilibrium concentration in the airlift reactor.

Volumetric oxygen transfer coefficient (K_La) and mixing time (t_m) estimation

The superiority of ALR for growth-associated PHB production over STR was further validated by estimating the volumetric mass transfer coefficient (K_La) and mixing time (t_m). Table 2 presents a comparison of ALR and STR in terms of K_La and t_m at optimized agitation and aeration rate of 200 rpm and 2 vvm, respectively. ALR with in situ cell retention was found to exhibit high oxygen transfer capacity (high K_La) in this investigation, particularly due to definite movement of air bubbles which reduces bubble coalescence and thereby increases the bubble residence time in the reactor and better bulk mixing, primarily due to its very specific design [7]. It is also noteworthy to mention here that the high oxygen mass transfer rate achievable in ALR along with the lower power consumption will lead to significant reduction in production cost [11]. The mixing criterion is used to maintain homogeneity inside the bioreactor, in order to obtain an optimal mass transfer, to avoid gradients of any of the medium components, and to keep the microcarriers or cells in suspension. Since mixing time is a useful parameter for assessing mixing efficiency and is applied to characterize bulk flow in reactors, a mixing time criterion was also used to compare the efficiency of STR and ALR in terms of PHB production. The

Table 2 Comparison of volumetric mass transfer coefficient and mixing time in ALR and STR at 200 rpm and 2 vvm

Reactor configuration	K_La (s^{-1}) at 2 vvm	Mixing time (s)
Stirred tank reactor	0.005	4.95 ± 0.03
Airlift reactor	0.016	3.73 ± 0.04

mixing time of both reactors was also different, with a shorter mixing time for ALR, further reinforcing the better mixing in ALR (Table 2). The superiority of ALRs in general over mechanically stirred reactors in terms of mass transfer rates for given energy input has also been demonstrated by Lamare and Legoy [16]. Thus, the high oxygen mass transfer coefficient and lower mixing time (Table 2) along with the cell settling can be considered as factors contributing to the enhanced performance of the ALR for growth-associated PHB production in this investigation as compared with STR. This characteristic of ALR will be very useful for fed-batch cultivation, which invariably features oxygen limitation. The results of the present study have demonstrated that ALR can also be used for PHB production at large scale because of lower installation and operation costs.

The volumetric mass transfer coefficient (K_La) is the most often applied physical scale-up variable and needs to be kept constant upon scale-up to maintain similar mass transfer of oxygen at larger production scales. It includes relevant parameters that influence oxygen supply, such as agitation and aeration [1]. One way of keeping the same K_La value as at the small scale is to improve power input by increasing the number of Rushton impellers within the large-scale reactor or increasing the agitation speed. However, in case of ALR (which lacks a rotating shaft and impellers), this operating variable cannot be manipulated to achieve similar K_La values at the larger scale. Hence, manipulation of aeration or air flow rate will be useful in maintaining a constant K_La upon scale-up. Therefore, in case of ALR, the sparging rate can be changed to achieve common K_La values at the two scales of bioreactor. Increasing the sparging rate would also help in maintaining a constant mixing time at the larger scale.

Conclusions

The present study has demonstrated the applicability of this novel airlift reactor with in situ cell retention for growth-associated PHB production. ALR was found to be better suited for PHB production because of lower energy demand, simple construction, easy operation, better aseptic control, and cell retention, with significantly higher PHB yield and productivity. The ALR exhibited a total of 30 and 43 % enhancement in biomass and PHB concentration, respectively, as compared with STR. Thus, ALR with cell retention could be an appropriate alternative for economic production of PHB on several counts, i.e., elimination of oxygen limitation, maintenance of higher cell density, and shorter mixing time, thereby leading to an overall PHB production rate of 0.84 g/h. It can even be scaled up to larger-scale bioreactors for mass production of biopolymers. It may also be noted that this is the first report on use of ALR (outer aeration and internal settling) with in situ cell retention for growth-associated PHB production by *A. australica*.

Acknowledgments The senior research fellowship (SRF) awarded by The Department of Biotechnology (DBT), Govt. of India, New Delhi for project execution is gratefully acknowledged by one of the authors (G.G.).

References

1. Alam Z, Muhd NH, Razali F (2005) Scale-up of stirred and aerated bioengineering bioreactor based on constant mass transfer coefficient. J Teknologi 43:95–110
2. Anderson AJ, Dawes EA (1990) Occurrence, metabolism, metabolic role, and industrial uses of bacterial polyhydroxyalkanoates. Microbiol Rev 54:450–472
3. Barham PJ, Barker P, Organ SJ (1992) Physical properties of poly (hydroxybutyrate) and copolymers of hydroxybutyrate and hydroxyvalerate. FEMS Microbiol Lett 103:289–298
4. Birch JR, Lambert K, Thompson PW, Kenney AC, Wood LA (1987) Antibody production with airlift fermentors. In: Lydersen BK (ed) Large scale cell culture technology. Hansen, New York, pp 1–20
5. Blakebrough N, Sambamurthy K (1966) Mass transfer and mixing rates in fermentation vessels. Biotechnol Bioeng 8:25–42
6. Braunegg G, Bogensberger B (1985) Zur Kinetik des Wachstums und der Speicherung von Poly-D (-) - 3-hydroxybuttersäure bei *Alcaligenes latus*. Acta Biotechnol 5:339–345
7. Chisti MY, Moo-Young M (1987) Airlift reactors: characteristics, applications and design considerations. Chem Eng Comm 60: 195–242
8. Chisti Y, Moo-young M (1988) Prediction of liquid circulation velocity in airlift reactors with biological media. J Chem Tech Biotechnol 42:211–219
9. Choi J, Lee SY (1999) Factors affecting economics of polyhydroxyalkanoate production by bacterial fermentation. Appl Microbiol Biotechnol 51:13–21
10. Dominguez A, Couto RS, Sanroman A (2001) Amelioration of ligninolytic enzyme production by *Phanerochaete chrysosporium* in airlift bioreactor. Biotechnol Lett 23:451–455
11. Fontana RC, Polidoro TA, da Silveira MM (2009) Comparison of stirred tank and air lift bioreactor in the production of polygalacturonase by *Aspergillus oryzae*. Bioresour Technol 100:4493–4498
12. Gavrilescu M, Tudose RZ (1998) Concentric-tube airlift bioreactors. Part 1: effects of geometry on gas hold-up. Bioprocess Eng 19(1):37–44
13. Gupta K, Mishra PK, Srivastava P (2009) Enhanced continuous production of lovastatin using pellets and siran supported growth of *Aspergillus terreus* in an airlift reactor. Biotechnol Bioprocess Eng 14:207–212
14. Hängii UJ (1990) Pilot scale production of PHB with *Alcaligenes latus*. In: Dawes EA (ed) Novel biodegradable microbial polymers. Kluwer, Dordrecht, pp 65–70
15. Horwitz W (1980) Official methods of analysis of the association of official analytical chemist. AOAC Methods, Washington
16. Lamare S, Legoy M (1993) Biocatalysis in gas phase. Trends Biotechnol 11:413–419
17. Lee SY (1996) Plastic bacteria? Progress and prospects for polyhydroxyalkanoates production in bacteria. Trends Biotechnol 14:431–438

18. Loo CY, Sudesh K (2007) Polyhydroxyalkanoates: bio-based microbial plastics and their properties. Malaysian Polym J 2: 31–57
19. Miller GL (1959) Use of dinitrosalicylic acid reagent for determination of reducing sugar. Anal Chem 31:426–428
20. Plackett RL, Burman JP (1946) The design of optimum multifactorial experiments. Biometrica 33:305–325
21. Riis V, Mai W (1988) Gas chromatographic determination of poly β-hydroxybutyric acid in microbial biomass after hydrochloric propanolysis. J Chromat 445:285–289
22. Saravanan P, Pakshirajan K, Saha P (2008) Performance of batch stirred tank bioreactor and internal loop airlift bioreactor in degrading phenol using *Pseudomonas* spp.: a comparative study. J Environ Protect Sci 2:81–86
23. Tavares LZ, da Silva ES, da Cruz Pradella JG (2004) Production of poly(3-hydroxybutyrate) in an airlift bioreactor by *Ralstonia eutropha*. Biochem Eng 18:21–31
24. Wang F, Lee SY (1997) Poly (3-hydroxybutyrate) production with high productivity and high polymer content by a fed-batch culture of *Alcaligenes latus* under nitrogen limitation. Appl Environ Microbiol 63:3703–3706
25. Wang SJ, Zhong JJ (1996) A novel centrifugal impeller bioreactor. II. Oxygen transfer and power consumption. Biotechnol Bioeng 51:520–527
26. Yezza A, Halasz A, Levadoux W, Hawari J (2007) Production of poly-β-hydroxybutyrate (PHB) by *Alcaligenes latus* from maple sap. Appl Microbiol Biotechnol 77:269–274
27. Yu PHF, Chua H, Huang AL, Lo WH, Ho KP (1999) Transformation of industrial food wastes into polyhydroxyalkanoates. Water Sci Technol 40:365–370