J Biochem Tech (2012) 4(1): 512-517
ISSN: 0974-2328

Scale up production of polyhydroxyalkanoate (PHA) at different aeration, agitation and controlled dissolved oxygen levels in fermenter using *Halomonas campisalis* MCM B-1027

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Received: 05 September 2012/Received in revised form: 18 October 2012, Accepted: 5 June 2013, Published online: 30 June 2013

Abstract

Production of biodegradable plastic, polyhydroxyalkanoate (PHA) was demonstrated using culture of moderately haloalkalitolerant *Halomonas campisalis* MCMB-1027. Production of PHA was carried out at different aeration (0.5-1, 1, and 1.5 vvm) and agitation (100,150 and 200 rpm) in 14 L fermenter. Maximum production of PHA was 40.69% on the basis of dry cell mass at 1 vvm and 100 rpm. Material balance over maltose revealed that aeration of 1 vvm and agitation of 100 rpm was optimum for conversion of utilized maltose into PHA. Controlled dissolved oxygen in the range 1-5% level during PHA accumulation phase facilitated increase in PHA content to 56.23%. A correlation equation was developed by fixing mass transfer coefficient (K_La) and applied successfully for scale up production of PHA in 120 L fermenter. ^{1}H NMR analysis showed percentage of HB and HV unit 95.83 and 4.17 respectively. Melting temperature was 166.5 ºC using differential scanning calorimeter (DSC). Gel permeation chromatography (GPC) revealed number average molecular weight ($\overline{M_n}$), weight average molecular weight ($\overline{M_w}$) and polydipersity index ($PI = \overline{M_w} / \overline{M_n}$) as 1.66 x 10^6, 2.08 x 10^6 and 1.25 respectively.

Key words: *Halomonas campisalis*, aeration, agitation, controlled dissolved oxygen, polyhydroxyalkanoate, scale-up production.

Introduction

The polyhydroxyalkanoates (PHAs) are environmentally important biopolymers of microbial origin. These biopolymers are accumulated intracellularly by a variety of microorganisms as carbon and energy reservoir under unbalanced nutritional and environmental conditions (Anderson and Dawes 1990; Wang and Lee 1997).

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PHA possesses important properties of thermoplasticity, biocompatibility and biodegradability. There is a need for development and production of polymer which is biodegradable, nontoxic and yet possesses the properties of synthetic polymer. PHAs have potential applications as disposable bulk material in packaging film, container, paper coating, bags, biodegradable carrier of herbicide, insecticide, fertilizer, and many more (Quillaguaman et al. 2007). PHA has medical applications such as stent, patches, sutures, vascular grafts etc. (Kulkarni et al. 2010).

Many researchers have demonstrated the role of dissolved oxygen concentration in PHA synthesis from different microorganisms. Nath et al. (2008) demonstrated scale up PHB production from *Methylobacterium* sp. ZP 24 in 30 L fermenter using limiting DO conditions resulted with 4.58 fold increase in production. Limitation of available oxygen inhibits cell growth and PHB accumulation in *Bacillus mycoides* RLJ B-017 (Borah 2002). Oxygen limitation (1-4% of air saturation) leads to increase in PHA content in *Halomonas boliviensis* as reported by Quillaguaman et al. (2007). Humphrey (1998) reported that exponents in the correlation equation vary with scale and medium of production. The value of proportionality constant of correlation equation which may be change depending upon the specific process (Beth et al. 2004).

Recently, we reported the production of PHB-co-PHV copolymer by moderately haloalkalitolerant *Halomonas campisalis* MCM B-1027, isolated from Lonar Lake, India. This organism accumulates PHA in the range of 45-81% when grown in production medium containing maltose and yeast extract as carbon and nitrogen source respectively (Kulkarni et al. 2010). High PHA accumulating bacterial species is considered to be good candidate for large scale production. We have also reported the characterization of this polymer, its biodegradability and potential application as a packaging material (Kulkarni et al. 2011).

This paper demonstrates the effect of aeration and agitation on distribution of oxygen uptake rate (xQo_2), volumetric mass transfer coefficient (K_La); effect of controlled dissolved oxygen level on scale up production of PHA from *H. campisalis*.

Materials and Methods

Culture

Halomonas campisalis isolated from the alkaline soda Lake of Lonar, District Buldhana, Maharashtra, India, was used for production of PHA. The culture is from MACS collection of microorganism (MCM, WDCM code 561) with culture No. MCM B-1027. The culture was maintained at 4 °C on nutrient agar (pH 9) supplemented with 3% (w/v) sodium chloride (Kulkarni et al. 2010).

Preparation of inoculum

The seed inoculum of *H. campisalis* was prepared by growing the culture in 400 ml nutrient broth of pH 9.0 supplemented with 3 % (w/v) NaCl, at 37°C for 18 h. After appropriate incubation, the culture broth was centrifuged at 7000 rpm for 30 min at 10°C (Kubota, Japan). The cell pellet obtained was washed with sterile saline, suspended in sterile saline and the cell density was measured spectrophotometrically (Shimadzu, Japan) at 600 nm. The optical density of the culture was adjusted to 1.70± 0.05 corresponding to 10^9 cells/ ml.

Production medium

1 L of production medium (Kulkarni et al. 2010) was prepared with minor modification as 10 g maltose, 1g yeast extract, 5 g NaCl, 0.2 g NaBr, 0.38 g $MgSO_4.7H_2O$, 0.128 g $CaCl_2.2H_2O$, and 0.75 g KCl. The production medium was sterilized *in situ* for 30 and 37 min in 14 and 120 L SS fermenters respectively. Maltose and calcium chloride were autoclaved separately (121°C for 15 min.) and transferred aseptically into the fermenters. The initial pH of the production medium was adjusted to 9.5 with 5M NaOH. All the chemicals used were of analytical grade.

Fermentation conditions

The production medium with initial pH of 9.5 was inoculated with 4 % (v/v) inoculum and incubated at 30°C for 24 h for all the experiments. Positive gauge pressure was maintained at 0.5 bar to avoid contamination. Agitation rates were varied in the range of 100 to 200 rpm and aeration rates were changed from 0.5 to1.5 vvm. Controlled dissolved oxygen (DO) experiments were carried out to enhance the PHA content by maintaining DO level at 1-5% or 10-15% after 12 h of fermentation.

Experimental set up

All the experiments were carried out in a 14 and 120 L stainless steel (SS) fermenters (Biochem Engineering Pvt. Ltd. Pune). Agitation was provided with fixed blade disc impeller. Air flow was controlled through rotameter. Oxygen uptake and oxygen transfer were measured during PHA production at constant temperature. Process temperature was maintained constant by circulating steam and cold water on jacket side of the fermenter. DO, pH, temperature and agitation were monitored over the period of fermentation by Supervisory Control and Data Acquisition (SCADA) software**.**

Batch fermentation

Production medium was inoculated with 4% (v/v) inoculum. Samples from fermenter were drawn after every 6 h and analysed for cell growth, maltose content, and qualitatively for PHA production. At the end of fermentation, the broth was centrifuged by continuous centrifuge (Model –AS 16 Y open type, Pennwalt India Ltd), cells were harvested and lyophilized (Model- ModulyoD-230, Thermo Electron Corporation, USA). All the results were average of two runs carried out independently in the fermenter.

Extraction and estimation of PHA

With the minor modification in the method demonstrated by Ramsay et al. (1994) for recovery of PHB and concentration of recovered PHA solution using rotary film evaporator was carried out in this investigation. PHA in the *Halomonas* cells was extracted in hot chloroform by using rotary film evaporator (Goel process systems, Pvt. Ltd, India) at 50°C and under vacuum within 3 h. Percentage ratio of dry cell weight (DCW) to chloroform was 0.338. A film was drawn by solution caste technique and weighed. PHA content (% PHA) was expressed with respect to dry cell weight.

Characterization of PHA film

PHA formed by *H. campisalis* was characterized for melting temperature, composition of monomer units and molecular weight.

Differential Scanning Calorimetric (DSC) analysis

Purified PHA film (~ 5 mg) was encapsulated in aluminum pans and heated in a temperature range from 50 to 200 ºC at the rate of 10º C/min using DSC, Perkin Elmer, USA. Melting temperature was recorded at the peak of melting endotherm.

^{1}H NMR Analysis

^{1}H NMR analysis of PHA film was done using Bruker NMR spectrophotometer, at 24ºC in $CDCl_3$.

Molecular weight determination by Gel Permeation Chromatography (GPC)

Molecular weight of the PHA formed was determined by GPC (Thermoquest). Chloroform was used as a mobile phase and polystyrene as a molecular weight standard.

Determination of xQo_2 and K_La in fermenter by dynamic gassing out method

Dynamic gassing out method by Taguchi and Humphrey (Senthilkumar et al. 2008) was used for measurement of oxygen uptake rate (OUR), oxygen transfer rate (OTR) and K_La in the fermenter using polarographic DO probe at every 6 h interval. The mass balance equation for dissolved oxygen (DO) in batch reaction can be expressed as,

$$\frac{dC_L}{dt} = K_L a(C^* - C_L) - xQo_2 \quad (1)$$

General form of Eq. (1) is,

$$X = Y - Z \quad (2)$$

where, Y is the oxygen transfer rate (OTR) and Z is oxygen uptake rate (OUR) of the culture. The measurement of OUR and OTR can be made in two stages.

In the first stage, inlet of airflow to the fermenter broth was blocked, and decrease in DO concentration due to respiration of microbial population was observed, which was sensed by dissolved oxygen probe. The volumetric OUR was determined by noting the changes

in DO concentration when oxygen flow was stopped. Equation (1) can be reduced to,

$$\frac{dC_L}{dt} = -xQo_2 \quad (3)$$

In the second stage, oxygen flow to the broth was restarted (agitation, air flow rate and pressure at predetermined values) and this led to increase in the DO concentration. Eq. (1) can be rearranged as,

$$C_L = -\frac{1}{K_L a}\left\{\left(\frac{dC_L}{dt}\right) + xQo_2\right\} + C^* \quad (4)$$

Thus from Eq. (4) a plot of C_L vs. $dC_L/dt + xQo_2$ will be a straight line with slope, $-1/K_L a$. Experiments were carried out to determine OUR and $K_L a$ at different stages of growth such as lag phase, exponential phase and stationary phase.

Analytical Methods

DO and pH Measurement

Dissolved oxygen (DO) was monitored *in situ* by using sterilizable polarographic oxygen sensor (Model-D400-B070-PT-D9) and pH was monitored using sterilizable pH probe (Model-F635-B120-DH, Broadly James Corporation, USA).

Biomass and Maltose Estimation

UV-visible spectrophotometer (Shimadzu, Japan) was used to measure optical density of fermented broth at 600 nm. Maltose in the fermented broth was estimated by the DNSA method (Quillaguaman et al. 2005).

Results and Discussion

Effect of Aeration on xQo_2 and $K_L a$

xQo_2 and $K_L a$ values were determined at different aeration rates of 0.5-1.0, 1.0, and 1.5 vvm at agitation 100 rpm. Typical trends of oxygen uptake and transfer, respiration rate and $K_L a$ determination plots obtained for *H. campisalis* are shown in Fig. 1 (a), (b) and (c) respectively. The trends of distribution of xQo_2 and $K_L a$ values are shown in Fig. 1 (d) and (f) respectively. Increasing aeration from 0.5 to 1 vvm increased value of xQo$_2$ from 47.43 mg/L/h to 53.55 mg/L/h at 12 h of fermentation. The difference in the uptake rate was due to increase in microbial population at 1 vvm from that at 0.5-1 vvm. The values of xQo_2 decreased from 53.55 to 28.35 mg/L/h when the aeration was increased from 1 to 1.5 vvm at 12 h of fermentation. For the estimation of $K_L a$, the typical level of % DO for different aeration 0.5-1, 1 and 1.5 vvm, decreased in the range of 100-54, 100-62 and 100-64 % respectively. DO level before re-aeration was maintained at > 10 % of air saturation i.e. critical DO level (Senthilkumar et al. 2008). This is the DO level value which ensures that microbial activity is not affected due to lack of oxygen. Fig. 1 (f) indicates that $K_L a$ values increased when aeration was increased from 0.5-1 vvm to 1.5 vvm over the period of fermentation cycle. The decrease in $K_L a$ values at 12 h was due to the increase in microbial population. Trends of xQo_2 and $K_L a$ were similar in all the experiments. Maximum DCW of 1.14 g/L and PHA content of 40.69% were obtained at aeration of 1 vvm (Fig.2 (a)). Borah et al. (2002) demonstrated increase in cell mass with increase in aeration from 0.8 to 4.3 g/L but PHB synthesis by *Bacillus mycoides* RLJ B-017 was suppressed at higher OTRs. Thus our finding differs from that reported by Borah et al.

Effect of Agitation on xQo_2 and $K_L a$

xQo_2 and $K_L a$ values were determined at different agitation rates (100,150 and 200 rpm) and aeration1.0 vvm. The trends of distribution of xQo_2 and $K_L a$ are shown in Fig. 1 (e) and (g). Fig. 1 (e) shows maximum xQo_2 value at 12 h of fermentation and afterwards it decreased till termination of fermentation. After inoculation in the fermenter at aeration 1 vvm and agitation at 200 rpm, values of xQo_2 and $K_L a$ could not be measured for this strain because oxygen uptake rate was very low and oxygen transfer rate was very high. Maximum production of 1.14 g/L biomass

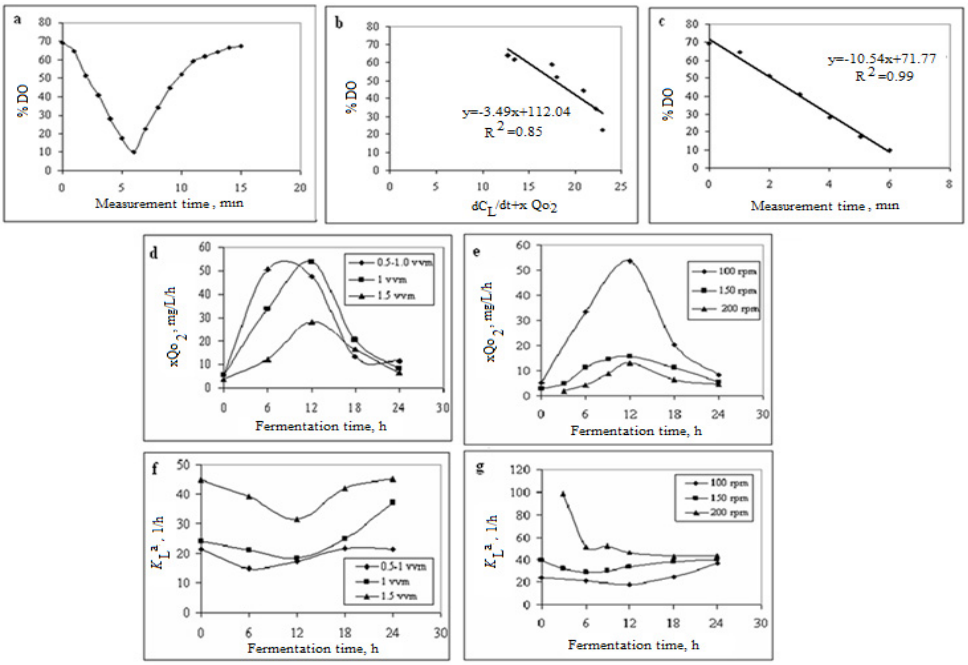

Figure 1: a) A typical trend of oxygen uptake and transfer during production of PHA b) Oxygen uptake rate by *Halomonas campisalis* (c) A plot of evaluating $K_L a$ (d) Effect of aeration on distribution of xQo_2 (e) Effect of agitation on distribution of xQo_2 (f) effect of aeration on distribution of $K_L a$ (g) effect of agitation on distribution of $K_L a$

containing 40.69 % PHA was achieved at 100 rpm (Fig. 2 (b)). Quillaguaman et al. (2007) reported that increasing rate of agitation increased PHA synthesis rate but reduced cell concentration. Thus our finding agrees with report of Quillaguaman et al. (2007).

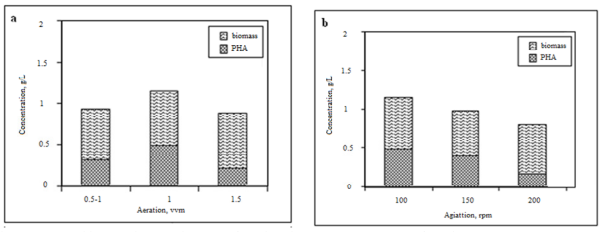

Figure 2: Effect of aeration and agitation on PHA production

Correlation of K_La, Impeller Speed and Superficial Air Velocity (V_s)

Optimum K_La, 18.42/h was observed at agitation rate of 100 rpm and aeration of 1 vvm. General equation (Senthilkumar et al.2008) correlating K_La, gassed power per unit volume (P_g/V) and superficial velocity (Vs) is,

$$K_L a = K(P_g/V)^a (V_s)^b \quad (5)$$

Values of exponents *a* and *b* are specific for each microorganism. Values of exponents were determined by EXCEL software. In the absence of measurement instrument for actual power required by impeller the exponent of (N^3D^2) was obtained as the representation of P_g/V, modified to following form equation (5) given below,

$$K_L a = K(N^3 D^2)^a (V_s)^b \quad (6)$$

The exponent *a* varied in the range of 0.07-0.49 (Fig. 3 (a)).Exponential relationship between K_La and impeller speed is given by equation,

$$K_L a \alpha (N^3 D^2)^{0.07-0.49} \quad (7)$$

Three air flow rates 0.5-1, 1 and 1.5 vvm and corresponding values of K_La for every 6 h of fermentation cycle were used to determine the exponent *b*. Because oxygen deficient condition was observed at 0.5 vvm, aeration was increased to 1 vvm after 9 h of fermentation. Exponential relationship is established between K_La and superficial velocity given by equation,

$$K_L a \alpha (V_s)^{0.51-0.85} \quad (8)$$

This investigation revealed that value of *b* varied in the range of 0.51-0.85 (Fig. 3 (b)). Minimum value of exponent *b* was 0.51 in the initial stage of stationary phase and maximum was 0.85 observed at middle of exponential phase. Exponent values were obtained for superficial gas velocity which was dependent on the ratio of liquid depth/ fermenter diameter, diameter of fermenter and aeration rate (i.e. volumetric air flow rate/ volume of medium).

Influence of Controlled Dissolved Oxygen on PHA Production

Two stage oxygen transfer strategy was used to improve the yield of biomass and PHA. The overall maximum value of PHA in dry cells, dry cell concentration, PHA concentration and volumetric productivity were 56.23%, 1.72 g/L, 0.97g/L, 0.04g/L/h respectively when DO was maintained at 1-5% of air saturation value. The organism needs oxygen in growth phase, and enhancement in PHA accumulation within cells was supported

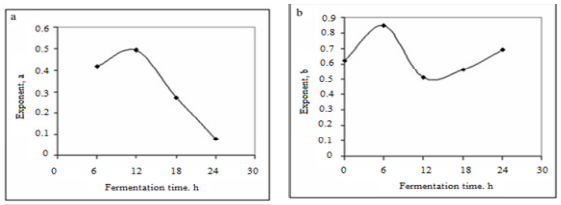

Figure 3: Dependency of K_La on impeller speed (a) and superficial gas velocity (b) during production of PHA in fermenter

by oxygen limitation. Experimental results obtained are shown in Table 1. Lower aeration during the PHA accumulation phase reduces cost of production. Similar strategy of oxygen limitation on PHB production was demonstrated by Quillaguaman et al. (2007) in 2 L fermenter by batch cultivation of *Halomonas boliviensis*. Oxygen limitation condition created by changing agitation speed to 4 % of air saturation resulted in increase in PHA content of from 44% to 50%. Quillaguman et al. (2005) also explained the mechanism how oxygen limitation condition is favorable for improvement of PHA content.

Lefebvre et al. (1997) showed that the overall productivity of fermentation was decreased and there was increase in concentration of 3-hydroxyvalerate monomer when the low dissolved oxygen was kept between 1-4% of air saturation. Wang and Lee (1997) divided PHA producing bacteria into two groups. The first group, which includes *Alcaligenus eutrophus,* Methylotrophs and Psuedomonads require limitation of an essential nutritional source for efficient synthesis of PHAs. The second group, which includes *Alcaligenus latus*, *Azotobacter vinelandii*, and recombinant *Escherichia coli* does not require nutrient limitation for PHA synthesis. The present studies indicate that *Halomonas campisalis* falls in the first group. Therefore our investigation confirms the report of Quillaguamn (2005) but differs from that of Lefebvre et al. (1997).

Choi et al. (2000) exhibited pilot scale production of P(3HB/V) in 30 and 300 L fermenter by fed batch culture of recombinant *Escherichia coli*.

Table 1: Effect of controlled and uncontrolled DO levels on maltose consumption and PHA production

Fermenter volume, L	% DO	% Maltose uptake	% (PHA /maltose uptake)	% (PHA/ total maltose)
14[a]	62-98	27.45	16.44	4.51
14[b]	10-15	34.72	18.61	6.46
14[c]	1-5	39.58	22.9	9.06
120[c]	1-5	37.1	17.3	6.41

14[a] - DO level not controlled after 12 h fermentation
14[b] - DO level controlled in the range of 10-15% after 12 h fermentation
14[c] and 120[c] - DO level controlled in the range of 1-5% after 12 h fermentation

Scale Up Production

Kulkarni et al. (2010) have demonstrated effect of different environmental parameters like inoculum density, incubation temperature, incubation period and initial pH of the medium on PHA production by *Halomonas campisalis* at flask level.

Maximum PHA content (69%) on dry cell weight basis and PHA concentration (1.1 g/L) was observed. Results achieved under this study are similar to production carried out in flask.

Table 2: Experimental and predicted values of K_La

Fermenter volume, L	Working volume, L	Aspect ratio	Experimental value of K_La, 1/ h	Predicted value of K_La, 1/ h
14	8	0.5	18.42	-
120	85	1.38	17.24	18.18

Oxygen transfer rate can control the overall rate of the bioprocess and volumetric mass transfer coefficient. For this reason the volumetric mass transfer coefficient has been preferred as the principal factor for scaling-up. Scale up in 120 L fermenter was done by fixing volumetric mass transfer coefficient (K_La) value. Value of exponents '*a*' and '*b*' at peak of oxygen uptake time were 0.49 and 0.5 respectively and specific factor *K* was 184.75. Experimental and predicted values of K_La are shown in Table 2 and results obtained from scale-up production are shown in Table 3. Lowering aeration and agitation rates from 1 to 0.21 vvm on scale-up production contributes to decrease in the cost of production. K_La value evaluated for 30 and 300 L fermenters were 0.11/sec i.e. 396/h and 0.03/ sec i.e. 108/h respectively (Choi et al. 2002). K_La value was not maintained at scale up production. Nath et al. (2008) demonstrated scale up PHB production from *Methylobacterium* sp. ZP 24 in 30 L fermenter by controlling the dissolved oxygen at fixed value i.e. 30 % of air saturation but no report on K_La. Humphrey (1998) reported that exponents in the correlation equation vary with scale and medium. *K* is proportionality constant whose value varies depending upon the specific process and the units of K_La, P_g/V_L and V_s (Beth et al. 2004). In this investigation value of K_La (18. 42/h), exponents (*a*-0.49 and *b*-0.5) and proportionality constant (*K* -184.75) were evaluated to control these values at 120 L fermenter. Puthli et al. (2005) studied the gas-liquid mass transfer with impeller combination with solution of sodium carboxymethyl cellulose and simulated fermentation medium in 2 L fermenter. They obtained values of K_La (0.0098/s which is equal to 35.28/h), exponents *a*-0.61 and *b*-0.43for dual impeller. Beth et al. (2004) reported scale-up methodologies for *Escherichia coli* and yeast fermentations by considering fixed exponents (*a*-0.67 and *b*-0.67) values to determine values of proportionality constant for fermentations of different microorganisms carried out at different scale. Senthilkumar et al. (2008) reported values of exponents (*a*-0.24 and *b*-0.41) for batch cultivation of *P. aeruginosa* in biocalorimeter. These results showed similar observations to those confirmed by Humphery (1998).

Maltose Consumption and PHA Production

Influence of different aeration rates on maltose uptake by H. campisalis and PHA production were investigated. Amount of maltose utilized by the organism and converted into PHA was determined by material balance over maltose. Maltose consumption increased with increase in aeration from 16.9 to 30.5 % but conversion of consumed maltose into PHA decreased with increasing aeration from 0.5 to 1.5 vvm as 17.15, 16.45 and 6.20 % respectively (Fig. 4 (a), (c), and (e)).At different rate of agitation (100, 150 and 200 rpm) conversion of consumed maltose to PHA was 16.45, 9.57 and 12.36 % respectively as seen in Fig. 4 ((b), (d), and (f)). Therefore aeration rate of 1.5 vvm and agitation rate 150 and 200 rpm decreased of conversion of consumed maltose into PHA. Aeration 1 vvm and agitation 100 rpm were optimum for conversion of consumed maltose into PHA.

Characterization of PHA

Characterization of film was done by using DSC, GPC and ^{1}H NMR. Melting temperature of PHA formed by *H. campisalis* was 166.5 ºC. Percentage of HB and HV fractions in PHA were 95.83 and 4.17 respectively indicating that the polymer is a copolymer of PHB-co-PHV. Number average molecular weight ($\overline{M_n}$), weight average molecular weight ($\overline{M_w}$) and polydipersity index ($PI = \overline{M_w} / \overline{M_n}$) were 1.66 x 10^6, 2.08 x 10^6 and 1.25 respectively.

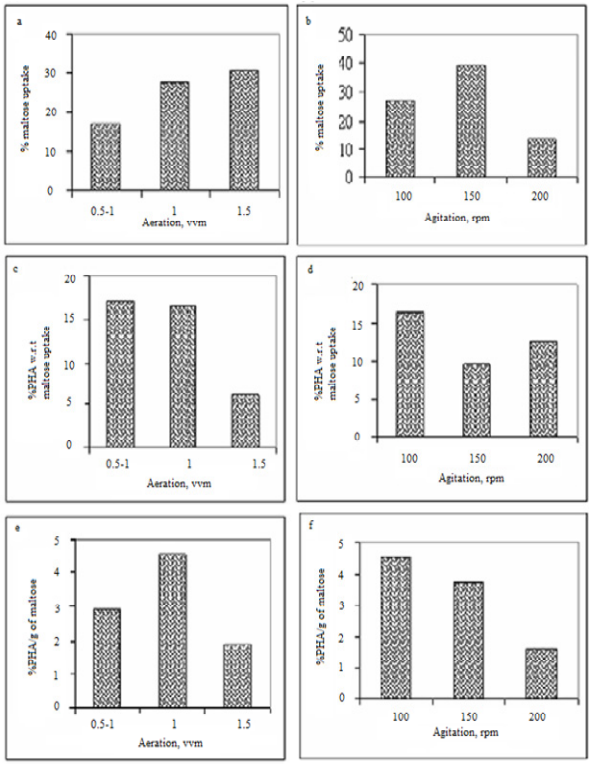

Figure 4: Effect of aeration rates [(a), (c) and (e)] and agitation rates [(b), (d) and (f)] on maltose uptake, % PHA based on maltose uptake and yield of PHA respectively

Conclusion

Experimentation carried out on influence of aeration, agitation rates and two stage oxygen transfer on production of PHA by *Halomonas campasalis* resulted in maximum PHA content 56.23% and PHA concentration of 0.97 g/L. The organism needs oxygen in growth phase and enhanced PHA accumulation within cells is supported by oxygen limitation. Controlling DO level during PHA accumulation phase reduces cost of aeration and consequently cost of the PHA production. Material balance over maltose revealed that aeration of 1 vvm and agitation at 100 rpm were optimum for conversion of consumed maltose into PHA. Volumetric oxygen transfer coefficient (K_La) dependency on agitation rate and superficial velocity was studied for this culture by estimating exponent '*a*' and '*b*'. Values of exponent '*a*' and '*b*' and proportionality constant *K* used to develop correlation equation and effectively applied for scale up production of PHA in 120 L SS fermenter. This investigation could be a useful step for improvement of PHA production using alternative strategies like fed-batch (fixed/variable volume/cyclic), semi-continuous culture, and cell recycle approach etc.

Acknowledgement

This research was supported by the Department of Biotechnology (DBT), Govt. of India, New Delhi.

Table 3: Scale up of production of PHA

Fermenter Volume L	Aeration vvm	Agitation rpm	Aspect ratio	% PHA	Dry cell conc. g/L	PHA conc. g/L	Productivity g/L/h
14[a]	1	100	0.5	42.06	1.14	0.48	0.02
14[b]	1 & DO (10-15%)	100	0.5	51.45	1.37	0.69	0.028
	1 & DO (1-5%)	100	0.5	56.23	1.72	0.97	0.04
120[c]	0.21 & DO (1-5%)	60	1.38	49.17	1.33	0.67	0.028

14[a] -DO level not controlled after 12 h fermentation;14[b] - DO level maintained 10-15% after 12 h fermentation ;14[c] and 120[c] -DO level maintained 1-5% after 12 h fermentation

Nomenclature

C_L : Dissolved oxygen concentration in fermented broth (mmoles/L)
C^* : Saturated dissolved oxygen concentration (mmoles/L)
t : Time (h)
dC_L/dt : Oxygen transfer rate (mmoles O_2/L/h)
x : Concentration of biomass (g/L)
Qo_2 : Specific respiration rate, (mmoles O_2/ grams of biomass/h)
xQo_2 : Oxygen uptake rate (mmoles O_2/L/h)
K_La : Volumetric oxygen transfer coefficient, (/h)
K : Proportionality constant
V_s : Superficial velocity (m/s)
N : Impeller rotation speed, rpm
D : Impeller diameter, m
P_g/V : Powered gas/ volume of fermented broth (w/L)

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