Poly(3-hydroxybutyrate) Production from Natural Gas by a Methanotroph Native Bacterium in a Bubble Column Bioreactor

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doi: 10.15255/CABEQ.2017.1263

Original scientific paper
Received: December 3, 2017
Accepted: March 19, 2018

Reducing the total cost of poly(3-hydroxybutyrate) (PHB) production as an attractive substitute for conventional petrochemical plastics still remains an unsolved problem. The aim of this research was the screening of PHB-producing microorganisms and selection of the best suitable medium for microbial growth and PHB production from methane. A new isolated methanotroph for PHB production from natural gas was studied in different media. After selection of the suitable medium, the effect of five process variables (content of nitrogen source, disodium hydrogen phosphate, methane to air ratio, seed age, and pH) on PHB production was investigated in a bubble column bioreactor. Also, hydrodynamic and mass transfer factors (flow regime, mixing time, gas hold up, and $k_L a$) were considered. At optimum operating conditions and engineering parameters in a bubble column, PHB content in the dried biomass reached 25 % w/w. The results showed that pH is the most important variable in the selected conditions.

Keywords:
bubble column bioreactor, gaseous substrate, poly(3-hydroxybutyrate), methanotroph synthesis of PHB

Introduction

Poly(3-hydroxybutyrate) (PHB), as a biodegradable, biocompatible and thermoprocessible biopolyester, has attracted the interest of bioresearchers[1–3]. PHB and other members of poly(hydroxyalkanoates) can be produced by various bacteria and archaea with a wide spectrum of application, from medicine[4] to food packaging[5] and agriculture[6], due to their similar properties to thermoplastics and elastomers[7]. However, the main problems, which limit their widespread application, are still high production cost and thermal instability[7]. The costs of PHB production mainly depend on substrate, culture condition, and downstream processing. Many studies have so far been conducted for overcoming this problem, including the usage of cheap substrates[8–11], modeling[12], proper experimental design[13,14], and development of new recovery methods[15–18], as well as large-scale[19] and high cell-density production of PHB in membrane bioreactor[20], optimization of PHA production[21], isolation of new PHB producers[22], and control of PHB production by various analytical methods[23].

Methane is a suitable substrate for production of PHB as it is plentifully available not only as a natural gas but also as biogas in oil-rich countries[24–29]. Recycled gas closed circuit culture system has been reported for the growth of bacteria from gaseous substrate[10]. In this system, the limitation of oxygen causes the formation of intermediates of the Krebs Cycle and even the PHB biosynthesis pathway, harming or even rendering unfeasible the formation of PHB[30]. Due to process flexibility, appropriate mass and heat transfer, appropriate gas dispersion, and directed circulation flow, loop bioreactors are applied to develop aerobic fermentation. The circulation creates appropriate mixing in all phases and provides good mass transfer[24,30].

Bubble columns are a type of loop bioreactor, which provide several advantages during operation and maintenance, such as high heat and mass transfer rates, compactness, and low operating and maintenance costs. Three-phase bubble column reactors can be widely employed in reaction engineering

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(i.e., in the presence of a catalyst) and in biochemical applications where microorganisms are utilized as solid suspensions in order to manufacture industrially valuable bio-products[24].

There is no comprehensive investigation on the design of experiments and process variables influencing PHB production in a bubble column. Study of parameters characterizing the operation and transport phenomena of bubble columns may lead to better understanding of hydrodynamic properties, heat and mass transfer mechanisms, and flow regime characteristics[25,27,28,31–33].

In this study, four possible media were compared for rapid growth of biomass and PHB production by a new isolated bacterium. After selection of the best medium, an L_8 Taguchi design was used to evaluate the impact of some process variables, including methane to air ratio, pH, and some key components of liquid medium on PHB production. Finally, the performance of a bubble column bioreactor was studied for PHB production.

Materials and methods

Chemicals

Birchwood xylan, azure chitin, and azure keratin were purchased from Sigma–Aldrich (St. Louis, MO, USA). Standard hydrogen and methane gases were purchased from GL Sciences (Tokyo, Japan). All the other chemicals were purchased from Wako Pure Chemicals (Kyoto, Japan) and Sigma-Aldrich.

Screening and selection of microorganisms

Twenty methylotrophic microorganisms were isolated/extracted from the soil samples collected from the south of Iran. Among them, three colonies showed the presence of PHB granules. Based on rapid growth and PHB production in partial limitation of nitrogen, plate no. 1 (784) was selected as the most promising PHB producer. It was characterized by 16S rRNA.

Culture medium

In this study, PHB production was considered in four culture media. In each medium, the bacteria were cultured in the same conditions. In the end, the culture medium with the greatest amount of PHB was chosen as the best medium for further experiments. The composition of selected culture media is shown in Table 1.

Fermentation conditions

A bubble column reactor was used for PHB production[24–29]. Length and diameter of the reactor were 300 and 30 cm, respectively (Fig. 1). The effective volume of reactor was approximately 200 L.

Table 1 – *Compositions of the selected media for PHB production in bubble column bioreactor by the isolated methanotroph*

Mineral	784	Choi	M3	Borque
NH_4Cl (g L^{-1})	0.5	–	–	–
$(NH_4)_2SO_4$ (g L^{-1})	–	1.0	1.5	1.5
KH_2PO_4 (g L^{-1})	0.5	1.3	1.3	1.3
K_2HPO_4 (g L^{-1})	0.7	–	–	–
$Na_2HPO_4{\cdot}7H_2O$ (g L^{-1})	–	4.0	4.0	4.0
$MgSO_4{\cdot}7H_2O$ (g L^{-1})	1.0	0.4	0.4	1.3
$CaCl_2{\cdot}2H_2O$ (mg L^{-1})	200	3.3	20	60
$FeSO_4{\cdot}7H_2O$ (mg L^{-1})	4	1.3	20	60
$MnCl_2{\cdot}4H_2O$ (μg L^{-1})	30	–	–	–
$MnSO_4{\cdot}H_2O$ (μg L^{-1})	–	100	490	1470
$ZnSO_4{\cdot}7H_2O$ (μg L^{-1})	100	130	260	780
$CuSO_4{\cdot}5H_2O$ (μg L^{-1})	–	40	800	240
$NiCl_2{\cdot}6H_2O$ (μg L^{-1})	20	–	–	–
$Na_2MoO_4{\cdot}2H_2O$ (μg L^{-1})	60	40	80	2.40
$COCl_2{\cdot}6H_2O$ (μg L^{-1})	200	40	800	240
H_3BO_3 (μg L^{-1})	300	30	600	180
$CuCl_2{\cdot}2H_2O$ (μg L^{-1})	10	–	–	–

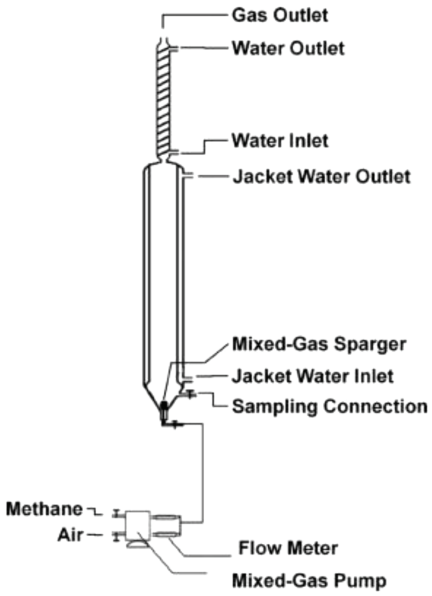

Fig. 1 – *A schematic diagram of the bubble column bioreactor applied for PHB production by the isolated methanotroph*

Flow regime and distribution of bubbles were completely uniform and homogenized. To avoid evaporation of the culture medium, a condenser was placed at the top of the reactor. A gas distributor sintered with low mesh was used to create bubbles of small diameter and uniform distribution. The fermentation process was performed in non-sterile conditions.

Bacterial growth measurement

The intensity of light absorbed by the bacteria was measured with a spectrophotometer at a wavelength of 600 nm. For measurement of cell dry weight, 3 mL of the fermentation culture medium was taken in sterile conditions at different times and diluted with distilled water.

PHB measurement

Polyhydroxybutyrate polymer was extracted using the dispersion method. Bacterial cells were collected by centrifugation at 10,000 rpm (radius of centrifugation of 5 cm) for 10 min at room temperature. The cell pellets were washed with phosphate buffer saline (pH 7.4). Then they were air-dried and their weights were recorded. Chloroform and sodium hypochlorite were added to the cell pellets in a ratio of 12.5 μL chloroform and 12.5 μL sodium hypochlorite per mg of pellet weight. The mixture was kept overnight at room temperature. It was then centrifuged at 8000 rpm for 10 min at room temperature, resulting in the formation of different phases. The bottom phase of chloroform containing PHB was transferred to another fresh tube and its volume was measured. Three-fold volume of methanol was added to the chloroform solution. The mixture was centrifuged at 10,000 rpm for 15 min, resulting in the formation of PHB precipitate. The amount of PHB present was then quantified by determining the weight of the obtained precipitate. The supernatant was discarded and the pellets were dissolved in boiling concentrated sulfuric acid, which gave a brown solution. The PHB content of biomass was determined by gas chromatography (GC-CP3800, Varian, USA) equipped with FID detector[34].

PHB-producing bacteria were further confirmed using Sudan Black B staining method. Sudan Black B stain was prepared as 0.3 % solution (w/v) in 60 % ethanol. The smear of cultures was made on glass slides and heat fixed. The samples were stained for 10 min with Sudan Black solution, rinsed with water, counter stained with 0.5 % safranin for 5 min, and observed under light microscope at 1000x magnification.

Flow regime

Several methods are available for estimation of flow patterns in gas–liquid flow. The best common way to identify different flow patterns is to observe the flow in a transparent channel or through an inspection window on the wall. Besides visual observation, photographic or videographic recording has also been extensively used. For observing a rapidly moving two-phase system, high-speed photography or videography is necessary. In the present work, photographic recording was done with a high-speed camera (Canon Powershot S3 IS), and the effect of aeration (vvm) rate on flow regime was investigated[32–37].

Mixing time

In most studies, mixing time (t_m) is considered as a function of circulation time; hence, it is better to control mixing time by aeration. It is commonly defined as the time required to realize a specific percentage of concentration homogeneity (95 % of its final value). In the present work, t_m was determined using tracer techniques in the presence of oxygen. This method is based on the fact that, if a pulse of tracer (a dye) is added to the flow, a decaying sinusoidal type of oscillation is perceived[32]. Tracer (0.5 mL, Brilliant Blue G, λ = 595 nm) was added to the bioreactor. Values of optical density were recorded in a spectrophotometer (PG instruments T60) until the oscillation response of the pulse was, finally, suppressed (each experiment was carried out in triplicate)[32].

Table 2 – *L_8 Orthogonal array for PHB production in a bubble column bioreactor by the isolated methanotroph*

Trial Number	Nitrogen source (A)	Na_2HPO_4 g L^{-1} (B)	pH C	Seed age (h) D	CH_4: air (E)	PHB accumulation %w/w
1	$(NH_4)_2SO_4$	0.00	7.5	36	30:70	17.5[d]
2	$(NH_4)_2SO_4$	0.00	8.5	48	50:50	19.5[f]
3	$(NH_4)_2SO_4$	4.02	7.5	48	50:50	25.5[g]
4	$(NH_4)_2SO_4$	4.02	8.5	36	30:70	13.5[b]
5	$NaNO_3$	0.00	7.5	36	50:50	15.5[c]
6	$NaNO_3$	0.00	8.5	48	30:70	11.0[a]
7	$NaNO_3$	4.02	7.5	48	30:70	18.5[e]
8	$NaNO_3$	4.02	8.5	36	50:50	15.5[c]

Gas holdup and mass transfer

Gas holdup (ε) was determined by the well-known method of volume expansion. The volume expansion within a zone in a bioreactor is calculated from the hydrostatic pressure difference between the two heads of a U-tube manometer attached above and below the zone, respectively. The gas holdup (ε) was measured with a high-speed camera (Canon Powershot S3 IS) as well. Gas holdup is identical to the ratio of volume expansion to ungassed volume. Volumetric mass transfer coefficient, $k_La(O_2)$, was determined by the "gas out-gas-in" method. The dissolved oxygen was first removed from the bioreactor by sparging with nitrogen until the dissolved oxygen concentration dropped to approximately zero, as indicated by the readings of the dissolved oxygen (DO) probe located within the bioreactor. DO was then stopped and sparging of air was restarted. The increase in DO was measured with time until the fluid became practically saturated with oxygen. The mass balance of DO in the bioreactor then gives Eq. (1)[32–37]:

$$dC_L/dt = k_La(C^* - C_L) \quad (1)$$

where, C_L is DO concentration, C^* is saturated oxygen concentration, and t is time.

Taguchi design

All the factors have been assigned only with two levels. Qualitek-4 Software was used for designing the experiments and data analysis. Preliminary experiments were conducted to choose the levels. Table 2 shows the layout of the L8 orthogonal array used in the present study. All combinations of the assigned parameters values were included in the trials.

Optimal conditions

Culture medium composition, temperature, pH, ionic strength, etc., are important factors affecting the efficiency of the fermentation process. Due to interaction, evaluating the impact of each of the mentioned factors is very difficult. To investigate the effect of methane to air ratio, pH, Na_2HPO_4 concentration, nitrogen source, and seed age (as important process variables) on PHB accumulation, a statistical design was applied. Correlation analysis was performed by SPSS (ver. 13) and Minitab (ver. 16). The design included 16 trials. All experiments were performed in a bubble column reactor. Data are expressed as mean±SD. Differences between the means were evaluated using one-way ANOVA, and differences among the treatment means were assessed using T^2-test when the variances were unequal. All differences were considered significant at $p \leq 0.05$.

Results and discussion

Screening and selection of microorganisms

Among the 20 methylotrophic microorganisms, three colonies showed strong evidence for the presence of PHB granules. Further testing for rapid growth and PHB-producing microorganisms (under partial limitation of nitrogen) led to the selection of culture no. 1 784 as the most promising bacterium. This bacterial isolate belongs to the well-known group of the pink-pigmented facultative methylotrophs. It is a rod shaped, Gram negative, and motile bacterium having catalase and oxidase activities. It is deposited in PBCC (Petrochemical Biotechnology Culture Collection) as PBCC6, and belongs to *Microbacterium* sp. It was characterized by 16S rRNA with the following gene sequencing:

GCTTAACACATGCAAGTCGAACGGTGAACACGGAGCTTGCTCTGTGGGATCAGTGGCGAACGGGTGAGTA
ACACGTGAGCAACCTGCCCCTGACTCTGGGATAAGCGCTGGAAACGGCGTCTAATACTGGATATGTGACG
TGACCGCATGGTCTGCGTCTGGAAAGAATTTCGGTTGGGGATGGGCTCGCGGCCTATCAGCTTGTTGGTG
AGGTAATGGCTCACCAAGGCGTCGACGGGTAGCCGGCCTGAGAGGGTGACCGGCCACACTGGGACTGAGA
CACGGCCCAGACTCCTACGGGAGGCAGCAGTGGGGAATATTGCACAATGGGCGCAAGCCTGATGCAGCAA
CGCCGCGTGAGGGACGACGGCCTTCGGGTTGTAAACCTCTTTTAGCAGGGAAGAAGCGAAAGTGACGGTA
CCTGCAGAAAAAGCGCCGGCTAACTACGTGCCAGCAGCCGCGGTAATACGTAGGGCGCAAGCGTTATCCG
GAATTATTGGGCGTAAAGAGCTCGTAGGCGGTTTGTCGCGTCTGCTGTGAAATCCGGAGGCTCAACCTCC
GGCCTGCAGTGGGTACGGGCAGACTAGAGTGCGGTAGGGGAGATTGGAATTCCTGGTGTAGCGGTGGAAT
GCGCAGATATCAGGAGGAACACCGATGGCGAAGGCAGATCTCTGGGCCGTAACTGACGCTGAGGAGCGAA
AGGGTGGGGAGCAAACAGGCTTAGATACCCTGGTAGTCCACCCCGTAAACGTTGGGAACTAGTTGTGGGG
TCCATTCCACGGATTCCGTGACGCAGCTAACGCATTAAGTTCCCCGCCTGGGGAGTACGGCCGCAAGGCT
AAAACTCAAAGGAATTGACGGGGACCCGCACAAGCGGCGGAGCATGCGGATTAATTCGATGCAACGCGAA
GAACCTTACCAAGGCTTGACATATACGAGAACGGGCCAGAAATGGTCAACTCTTTGGACACTCGTAAACA
GGTGGTGCATGGTTGTCGTCAGCTCGTGTCGTGAGATGTTGGGTTAAGTCCCGCAACGAGCGCAACCCTC
GTTCTATGTTGCCAGCACGTAATGGTGGGAACTCATGGGATACTGCCGGGGTCAACTCGGAGGAAGGTGG
GGATGACGTCAAATCATCATGCCCCTTATGTCTTGGGCTTCACGCATGCTACAATGGCCGGTACAAAGGG
CTGCAATACCGCGAGGTGGAGCGAATCCCAAAAAGCCGGTCCCAGTTCGGATTGAGGTCTGCAACTCGAC
CTCATGAAGTCGGAGTCGCTAGTAATCGCAGATCAGCAACGCTGCGGTGAATACGTTCCCGGGTCTTGTA
CACACCGCCCGTCAAGTCATGAAAGTCGGTAACACCTGAAGCCGGTGGCCTAACCCTTGTGGAGGGAGCC
GTCGAAGGTGGGATCGGTAATTAGGACTAAGTCGTAACA

Selection of the best medium for PHB production

To choose the best medium for PHB production, four culture media were considered. The fermentation process in all the selected media was performed in a bubble column reactor using natural gas as the substrate. Fig. 2 shows the value of optical density of the media at 600 nm. The decrease in the curve slope in the decelerating growth phase indicates the beginning of PHB accumulation phase. The measured PHB production rates in the mentioned media are summarized in Table 3.

The data related to OD (at a wavelength of 600 nm), biomass production, and PHB production are given in Table 3. It was supposed that the maximum amount of biomass and PHB production was obtained in the optimum culture medium. Therefore, this medium of Choi was selected as the main culture medium for further steps of research.

Table 3 – *PHB accumulation in different culture media in a bubble column bioreactor by the isolated methanotroph*

Culture media	PHB accumulation (% w PHB/w biomass)	OD at λ = 600 nm	Biomass g L^{-1}
784	10[a]	0.6	0.9
Choi	23[c]	1.8	2.2
M3	17[b]	1.2	1.7
Borque	16[b]	1.1	1.5

[abc] Means within a row with different lowercase superscripts differ mutually ($p \leq 0.05$).

Optimization of PHB production in a bubble column

The characterization of flow regime in the bioreactor was done by changing the aeration rate and observing the flow with the high-speed camera. The bubbly flow regime was detected, similarly to the other studies[32–37]. At optimum flow rate, the gas holdup was 0.08. Like the gas holdup, changes in the rate of aeration caused the same effects for $k_L a$. Main enhancement in $k_L a$ occurred at 0.09 vvm, and resulted in the value of 0.21 s^{-1}.

After selecting the best hydrodynamic and mass transfer parameters, and the appropriate culture medium for PHB production (Table 3), the culture medium composition and operating conditions were optimized. Nitrogen source, seed age, methane

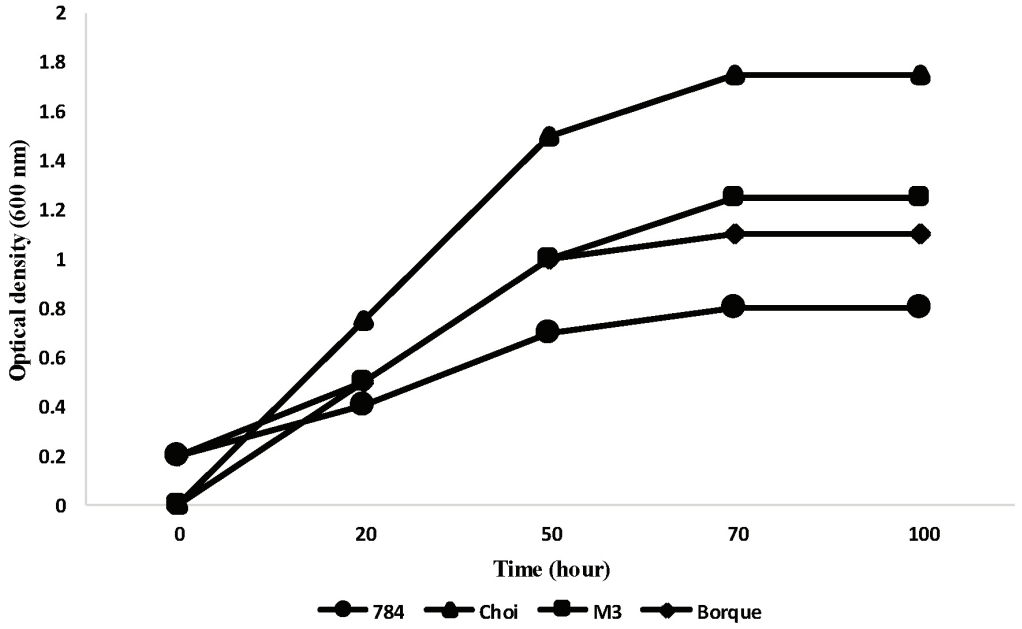

Fig. 2 – *Growth curve of the isolated methanotroph for PHB production in a bubble column bioreactor in different media*

to air ratio, Na_2HPO_4 concentration, and pH were the variables, whose influence on PHB accumulation was evaluated by statistical analysis. Analysis of variance (ANOVA) of the obtained experimental data is presented in Table 4, where the last column indicates the influence of each factor.

The pH was the most significant factor for PHB production, and nitrogen source and seed age showed a moderate effect on biopolymer production. The remaining factors (i.e., methane to air ratio, Na_2HPO_4 concentration, and pH) showed negligible influence on biopolyester production at individual levels. These results are similar to previous reports. Zhang *et al.* showed the effect of media composition and methane to air ratio on PHB accumulation[38,39]. Bourque *et al.* also investigated the effect of various fermentation conditions and nitrogen content on PHB accumulation[39]. Rahnama *et al.* reported the significant impact of methane to air ratio and nitrogen content on PHB production[24].

Effect of nitrogen source on PHB production

The effect of nitrogen source on the amount of PHB accumulation was studied. Ammonium sulfate and sodium nitrate salts were used as nitrogen sources. As Table 4 shows, the use of ammonium sulfate as a nitrogen source led to higher PHB accumulation. This observation is similar to the report of Khana *et al.*, who studied the impact of different carbon and nitrogen sources in culture medium on PHB production by *Ralstonia eutropha*[38,39].

Effect of Na_2HPO_4 concentration on PHB production

Here, Na_2HPO_4 was used as a source of phosphate. The high concentration of this salt causes higher PHB accumulation. Nevertheless, among all the studied variables, the concentration of Na_2HPO_4 showed the lowest impact on PHB accumulation. Asenjo *et al.*[43] examined the effects of magnesium

Table 4 – *Analysis of variance of Taguchi method applied for PHB production in a bubble column bioreactor by the isolated methanotroph*

Variable	Degree of freedom	Sum of squares	Variance	Variance ratio	Pure sum of squares	Effect (%)
Nitrogen content	1	60.062	60.062	73.923	59.25	21.868
Na_2HPO_4	1	18.062	18.062	22.23	17.25	6.366
pH	1	85.562	85.562	105.307	84.75	31.28
Methane to air ratio	1	3.062	3.062	48.076	38.25	14.117
Seed culture age	1	60.062	60.062	73.076	59.25	21.868
Error	10	8.124	8.124	–	–	4.501

and phosphate limitation on the distribution of PHB molecular weights by *R. eutropha*. In other studies, the decrease in magnesium content of the medium lowered the molecular mass of PHB, whereas phosphate had negligible impact on it[39–46].

Effect of methane to air ratio on PHB production

By using a gas mixture with the same amount of methane and air, a higher concentration of PHB was obtained. Wendlandt *et al.* studied the impact of gas mixture on PHB production in a rapid, non-sterile process by a methanotrophic strain *Methylocystis* sp[23]. Lafferty *et al.* investigated the effect of oxygen limitation on PHB amount by *R. eutropha*. Their results showed increased PHB production in partial limitation of oxygen[47].

Effect of seed age on PHB production

The effect of seed age on PHB production was examined at two levels of 36 and 48 h. PHB accumulation at 48 h was higher than at 36 h. Rahnama *et al.* also investigated the effect of seed age on PHB production. The results showed that the best time for high PHB accumulation was 84 h[24].

Effect of pH on PHB production

The effect of pH on the amount of PHB accumulation was studied. PHB accumulation at pH 6 was higher than at pH 7. Shimizu *et al.* investigated the PHB production by *R. eutropha*. Optimal conditions for PHB production by *R. eutropha* were at pH 6.9 and 7.5, and PHB accumulation was 53 % and 58 %, respectively[48].

After selecting the optimal point, an experiment was conducted in the optimized culture medium, as described in Table 5. The PHB content of 25 % w/w was obtained, which agrees well with the result predicted by the software. Gas chromatography was used to measure the amount of PHB accumulation.

Shah *et al.* applied *Methylosinus trichosporium* OB3b for PHB production under methane-dependent growth and batch conditions in a 4-L bioreactor. The PHB content was 10 %, while the bioprocess time was 140–150 h[44]. In addition, *Methylocystis parvus* OBBP was applied for PHB production in a shake flask by Asenjo and Suk[43]. Shaking speed and temperature were adjusted on 300 rpm and 30 °C, and PHB content reached the level of 70 % after 310 h of fermentation. Zhang *et al.* used methane and methanol as the carbon sources for PHB production. The PHB content of 40 % was produced by *M. trichosporium* IM V3011 during 144 h in a shake flask at 200 rpm and 30 °C[39]. Wendlandt *et al.* used methanotrophic strain

Table 5 – *Optimum conditions for PHB production in bubble column bioreactor by the isolated methanotroph*

Variable	Level (surface)	Description
Nitrogen source	1	$(NH_4)_2SO_4$
Na_2HPO_4	2	4.02 (g L^{-1})
pH	1	7.5
Seed age	2	48 h
Gas to air	2	50:50

Methylocystis sp. GB 25 DSMZ 7674 for PHB production from methane in a non-sterile process using 7-L and 70-L pressurized bioreactors[23]. Cultivation was performed in two stages: a continuous growth phase (dilution rate 0.17 h^{-1}) and a PHB accumulation phase under deficiency conditions of an essential nutrient (ammonium, phosphate, or magnesium) in the batch culture. The PHB content of biomass was as high as 51 %. Rahnama *et al.* studied PHB production by *Methylocystis hirsute* from natural gas in two different media. They investigated the impact of two key process variables (i.e., methane to air ratio and nitrogen content) on PHB production in a bubble column bioreactor using a full factorial design. It was found that both factors had significant effect on PHB accumulation. The PHB content of biomass was 51.6 % w/w of cell dry weight in the vertical tubular loop bioreactor[24]. The optimum conditions for each variable for the highest PHB accumulation are shown in Table 5. The data were analyzed using Qualitek-4 software. Kourmentza *et al.*[45] considered recent advances and challenges towards sustainable PHA production. Koller *et al.*[46] evaluated the production of microbial PHA biopolyesters in a sustainable manner, and Tan *et al.*[49] gathered and reviewed the main research works on biopolymer PHA.

Conclusion

In this study, a native methanotroph was used to produce PHB from natural gas in the bubble column reactor. Four possible media were compared for rapid growth and PHB production by a new isolated bacterium. After selection of the best medium, an L_8 Taguchi design was used to evaluate the impact of some process variables on PHB production. The performance of a bubble column bioreactor was studied for PHB production. The effect of five process variables (nitrogen source, Na_2HPO_4 content, methane to air ratio, seed age, and pH) on PHB production was investigated in a bubble column bioreactor. The results suggested pH as the most important variable in the selected conditions for

PHB production. PHB production was measured at optimum operating conditions and engineering parameters. PHB content in the dried biomass reached 25 % w/w.

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