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Enhancing Poly(3-hydroxybutyrate) Production through the Optimization of Reactor Geometry

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Poly(3-hydroxybutyrate) (PHB) is a thermoplastic polyester that can be synthesised by many bacteria under unfavourable growth conditions. Among all its properties, biodegradability is the most attractive since it makes PHB a potential alternative to non-biodegradable plastics. However, the current market penetration of this polymer is limited by the high production costs, which accounts for approximately 40% of the total price, and the complexity in scaling-up the biological process. In this work, an innovative approach is presented, simulating the production of PHB through a double stage aerobic fermentation in which a *Methylocystis* strain grows and accumulates PHB granules. The effects of the reactor geometry on PHB yields were investigated in terms of aspect ratio (A), which was varied in the range 5-16 both at constant volume and variable volume: in the first case, the working volume was kept constant at 100 L and the diameter of the reactor was varied in the range 0.2-0.3 m; in the second case, the working volume was varied between 100 and 340L, keeping constant the diameter at 0.3 m. The effect of the geometry on transition parameters was evaluated as well. Results show that a maximum transition superficial gas velocity (U_t) of 0.05 ms^{-1} was obtained at A=16; while the productivity of PHB ranged between 0.12 and 0.25 $kgm^{-3}d^{-1}$.

1. Introduction

Fossil-based plastics are considered something that is destroying the planet since the disposal of single-use products has detrimental effects on the environmental matrices, affects the ecosystem and is responsible for health-related problems. Due to all the above-mentioned problems, today, the attention is addressed to discovering an effective solution to plastic pollution. Poly(3-hydroxybutyrate) (PHB) is a biobased and biodegradable polymer that shows good barrier properties to gases and water, is highly crystalline (Padermshoke et al., 2005), stiff but also relatively brittle (Doi et al.1995) and can be produced biologically by many microorganisms when the macronutrients essential for their growth are not included in the culture medium (Koller et al., 2010). However, despite the attractive properties, the PHB market spread is still hampered by the high production costs, the 40% being related to the expensive carbon sources employed in the process (Khosravi-Darani et al., 2013). Moreover, for PHB to replace fossil-based plastics, it is necessary to ensure high production capacities. In this context, design and scale-related effects in bioreactors are not negligible and should be carefully evaluated since they may have a strong influence on the viability of the biomass (Besagni et al., 2019). For instance, if the aeration rate increases beyond a certain limit, the turbulent flow may occur, and the shear stress caused by the high superficial gas velocity (U_g) can damage the cell structure and reduce the concentration of the living biomass (Toma and Systems, 1991). Since this phenomenon, known as turbohypobiosis, may adversely affect processes involving bacteria (Rikmanis et al., 2007), such as PHB production, the fluid dynamics of the system should be controlled to avoid a turbulent regime. In bubble column reactors, which are commonly used bioreactors (Besagni et al., 2018), turbulent conditions are represented by the so-called “heterogeneous regime”, which starts when the transition gas hold-up (ε_t) and superficial gas

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velocity (U_t) are overcome (Manjrekar and Dudukovic, 2019). Therefore, to guarantee the permanence of the homogeneous regime (Figure 1), a proper estimation of the transition parameters is required.

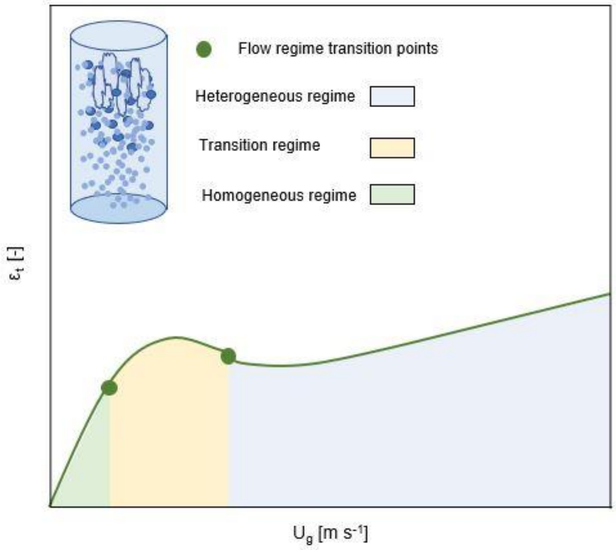

Figure 1: Flow regimes in bubble columns

When the reactor diameter (*D*), the height of the liquid (*H*) and the superficial gas velocity change, some alterations can be observed in flow regime, phase hold-up and mass transfer characteristics.
Many authors developed correlations for the calculation of ε_t and U_t (Urseanu, 2000), some of them relating these parameters to the geometry of the reactors (Ruzicka et al., 2001), such as the reactor diameter, the height of the liquid and the aspect ratio (*A*) (the ratio between the height of the liquid and the diameter of the reactor).
This study aims at analysing the PHB production from a cheap and renewable substrate, such as methane, emphasising scale and design effects. Three representative geometries were evaluated at constant and variable volumes. For each case studied, the transition parameters were estimated as a function of the geometry of the reactors to assure the homogeneous regime and high cell viability during the whole process.

2. Materials and Methods

This section describes the method used to simulate the process for producing Poly(3-hydroxybutyrate) from methane in bubble column bioreactors, which are commonly used reactors in biological processes, and the strategy used to enhance the process performance. All the data for the simulations, such as reactions, bacteria strain, kinetic parameters and duration of the process were taken from some experimental works reported in the literature.

2.1 Process setup

In this work, the process was simulated in AspenPlus following a double stage fermentation. Firstly, *Methylocystis parvus OBBP* was grown in a semicontinuous bubble column bioreactor under optimal metabolic conditions. A gas stream containing methane and oxygen (pCH_4=0.4 atm) was fed to guarantee aerobic conditions and the supply of a carbon source as well. After 10 days of cultivation, the grown biomass was moved to the second bioreactor for a 7 days Poly(3-hydroxybutyrate) accumulation. Here, the culture medium was nitrogen-deprived, but the carbon source was still supplied at 0.4 atm in a continuously fed gas stream. The process scheme is sketched in Figure 2.
After defining the flowsheet, a reaction was assigned to both reactors: the first for the growth of *Methylocystis parvus OBBP*; the second for the accumulation of the biopolymer (Eq.1 and 2). Data were collected from Rostkowski et al. (2013) and Myung et al. (2015) for the growth phase and the accumulation phase, respectively.

$$\frac{1}{4}CH_4+\left(\frac{1}{4}+\frac{f_e}{4}\right)O_2+\frac{f_s}{28}NO_3^-+\left(\frac{29}{28}f_s+f_e-1\right)H^+\rightarrow\left(\frac{1}{4}-\frac{5f_e}{28}\right)CO_2+\left(\frac{f_e}{2}+\frac{11f_s}{28}\right)H_2O+\left(\frac{f_s}{28}\right)C_5H_7O_2N \quad (1)$$

$$\frac{1}{4}CH_4+\left(\frac{1}{4}+\frac{f_e}{4}\right)O_2\rightarrow\left(\frac{1}{4}-\frac{4f_s}{18}\right)CO_2+\left(\frac{f_e}{2}+\frac{f_s}{3}\right)H_2O+\left(\frac{f_s}{18}\right)C_4H_6O_2 \quad (2)$$

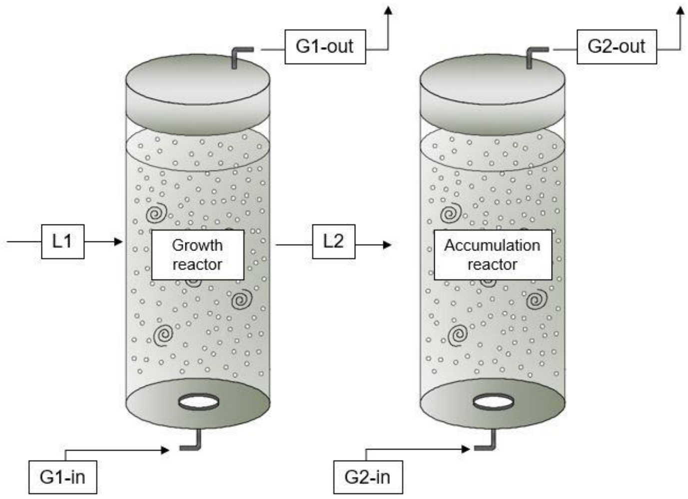

Figure 2: Process simulation flowsheet

2.2 PHB production optimisation

The effects of the geometry of the reactors on PHB yields were simulated using three representative conditions in terms of aspect ratio (low, medium and high aspect ratio), corresponding to *A*=5; 8; 16, which are in the range commonly reported in the literature (Table 2). For the first case study, three reactors with a working volume of 100 L and diameters of 0.2, 0.25 and 0.3 m were considered; while in the second group of simulations the diameter was 0.3 m and the volume varied between 100 and 340 L. Details of the cases are reported in Table 1.

Table 1 - Summary of the geometry configurations

Case study	*A* [-]	*D* [m]	*H* [m]	*V* [L]
	5	0.30	1.4	100
1	8	0.25	2.0	100
	16	0.20	3.2	100
	5	0.30	1.4	100
2	8	0.30	2.4	170
	16	0.30	4.8	340

2.3 Transition parameters estimation

As stated before, the performance of biochemical processes is affected by the hydrodynamics of the system, since high turbulence can impair the effectiveness of the process. Therefore, it is important to evaluate the condition that marks the passage from the homogeneous to the heterogeneous regime, identified through the transition gas hold-up and superficial gas velocity.
Ruzicka et al. (2001) proposed a theoretical equation (Eq.3) for calculating the transition gas hold-up that well matched the experimental results.

$$\varepsilon_g < \varepsilon_t = \frac{\nu k}{g}\frac{Ra_c^{\infty} + f(A)}{H^3} \tag{3}$$

where *f(A)* is a function of the aspect ratio (Ruzicka et al., 2001).
The transition superficial gas velocity was estimated as a function of ε_g, as reported by Reilly et al. (1994) (Eq.4):

$$U_t = V_{small}\varepsilon_t(1-\varepsilon_t) \tag{4}$$

In Eq.4, V_{small} is the velocity of small bubbles and can be calculated according to Eq.5.

$$V_{small} = \frac{1}{2.84}\frac{1}{\rho_g^{0.04}}\sigma^{0.12} \tag{5}$$

After the assessment of ε_t and U_t for each case study, the superficial gas velocity was set below the transition value.

3. Results

This section reports the values of U_t and the results of the simulations in terms of biomass and PHB productivities (*BIO_{prod} and PHB_{prod}, respectively)*.

3.1 Transition gas velocity as a function of the reactor geometry

The transition superficial gas velocity was calculated according to Reilly et al. (1994) as a function of the transition gas hold-up, which depends on some geometrical features (*D, A*). The results of the calculation (Table 3) show that U_t increased with the aspect ratio, both when the volume was kept constant and when it was varied between 100 to 340L; however, by keeping constant the volume, the transition superficial gas velocity resulted slightly higher. A maximum value of 0.05 ms^{-1} was obtained with an aspect ratio of 16 (case study 1). Some data of the transition parameters were reported in the literature with regards to different geometries (Table 2). It can be noted that a difference has always been measured when working different configurations, thus implying the need for testing several geometries to find the one that allows to work at higher superficial gas velocities, and to obtain higher mass transfer rates.

Table 2 - transition parameters as function of the reactor geometry

A [-]	*D* [m]	*H* [m]	ε_t [-]	U_t [m s^{-1}]	Reference
5	0.630	1.90	0.098	0.036	(Urseanu, 2000)
5	0.380	1.95	0.137	0.048	(Urseanu, 2000)
10	0.240	2.40	0.176	0.032	(Besagni, 2021)
10	0.190	1.90	0.148	0.034	(Urseanu, 2000)
12.5	0.240	3.00	0.181	0.035	(Besagni, 2021)
12.6	0.174	2.20	0.081	0.023	(Urseanu, 2000)
15	0.240	3.6	0.183	0.039	(Besagni, 2021)

Note that the values estimated are consistent with the literature, in which U_t is often reported in the order of 10^{-3} ms^{-1} (Table 2). Moreover, since each case study was simulated using a superficial gas velocity lower than the transition value, the homogenous regime was respected.

Table 3: Superficial gas velocities comparison

Case study	*A* [-]	U_t [ms^{-1}]	U_g [ms^{-1}]
	5	0.030	0.027
1	8	0.041	0.036
	16	0.050	0.047
	5	0.030	0.027
2	8	0.036	0.034
	16	0.043	0.040

3.2 Biomass and PHB productivities as a function of the reactor geometry

The productivity of both biomass and PHB was evaluated as a function of the aspect ratio in order to assess the effect of the geometry of the reactors on process yields.
It can be observed that PHB_{prod} and BIO_{prod} tend to decrease with *A* (Figure 4 and 5) and, the higher the aspect ratio, the higher the gap between the two cases simulated. However, it appears that to parity of aspect ratio, higher productivities can be obtained when the volume is kept constant, and the diameter of the reactor is varied, with values of BIO_{prod} and PHB_{prod} in the ranges 0.07-0.15 kg $m^{-3}d^{-1}$ and 0.12-0.25 kg $m^{-3}d^{-1}$, respectively. Note that, to the author's knowledge, there are no similar studies with focus on the assessment of the reactor geometry during the production of PHB. However, the values achieved in this work are coherent with the theoretical productivities of $\approx 10^{-2}$ kg $m^{-3}d^{-1}$ of PHAs. Moreover, similar productivities were previously reported in the literature, thus being consistent with the results achieved experimentally. Rodríguez et al. (2020), for instance, obtained 0.04-0.06 kgPHAs $m^{-3}d^{-1}$ when producing PHAs from biogas in a bubble column bioreactor, despite the theoretical production resulted in 0.26 kgPHAs $m^{-3}d^{-1}$. Higher values where obtained by García-Pérez et al. (2018) in a bubble column bioreactor.

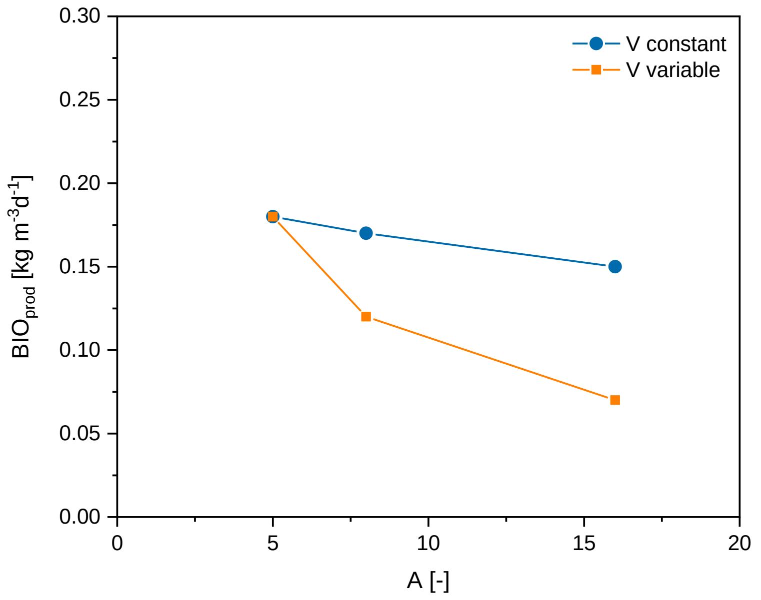

Figure 4: Productivity of biomass at constant volume (●) and variable volume (■) as a function of the aspect ratio

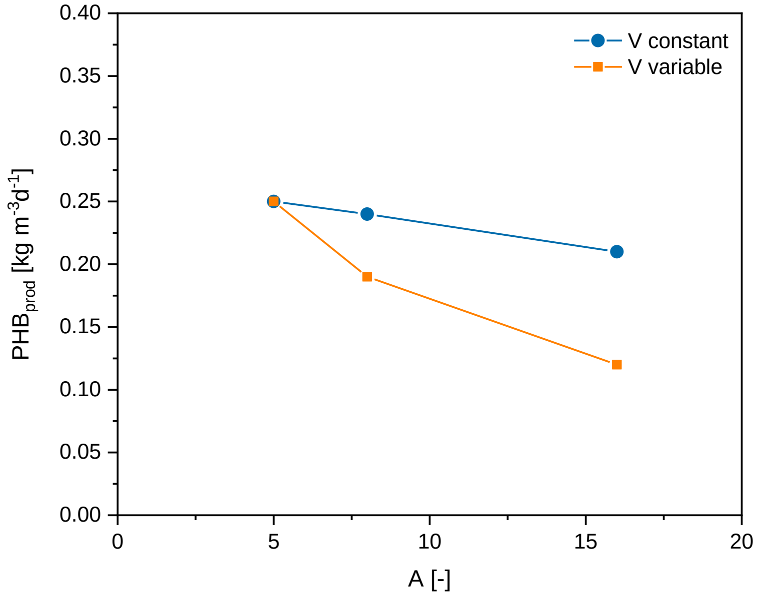

Figure 5: Productivity of PHB at constant volume (●) and variable volume (■) as a function of the aspect ratio

4. Conclusions

In this work, the Poly(3-hydroxybutyrate) production process was simulated, and the effect of some geometrical features on process yields was investigated. *Methylocystis parvus OBBP* was grown on nitrate for 10 days, and PHB was accumulated within 7 days in the absence of nitrogen in the culture medium. The process was simulated employing three couples of bioreactors with 100L capacity and variable aspect ratio (case study 1) and three couples of bioreactors with a constant diameter of 0.3 m and variable working volume (case study 2). The transition point was calculated for each case to assure that the hydrodynamic conditions fell into the homogeneous regime. Results showed that the transition superficial gas velocity grows with the aspect ratio, regardless of how it is increased (i.e., constant or variable volume). The productivities of biomass and PHB follow a downward trend when the aspect ratio is increased. Moreover, the higher the aspect ratio, the higher the gap between the two conditions considered. Furthermore, under the same aspect ratio, higher productivities can be achieved when the volume is kept constant, and the diameter of the reactor is varied.

Nomenclature

A - aspect ratio [-]
D – diameter [m]
d_b - bubble mean dimension [mm]
f_e, f_s - partition coefficients [-]
H - liquid height [m]
k - hydrodynamic diffusivity of the bubbles [m^2 s^{-1}]
pCH_4 – methane partial pressure [atm]
Ra_c^{∞} - critical Rayleigh number [-]
U_g - superficial gas velocity [m s^{-1}]
U_t - transition superficial gas velocity [m s^{-1}]
V – reactor volume m^3
V_{small} - velocity of small bubbles [m s^{-1}]
ε_g - gas hold-up [-]
ε_t - transition gas hold-up [-]
v - kinematic viscosity of the bubbly mixture [m^2 s^{-1}]
ρ_g - gas density [kg m^{-3}]
σ - superficial water tension [N m^{-1}]

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References

Besagni, G., 2021. Bubble column fluid dynamics: A novel perspective for flow regimes and comprehensive experimental investigations. Int. J. Multiph. Flow 135.
Besagni, G., Gallazzini, L., Inzoli, F., 2019. On the scale-up criteria for bubble columns. Petroleum 5, 114–122.
Besagni, G., Inzoli, F., Ziegenhein, T., 2018. Two-phase bubble columns: A comprehensive review. ChemEngineering 2, 1–80.
Doi, Y., Kitamura, S., Abe, H., 1995. Microbial Synthesis and Characterization of Poly(3-hydroxybutyrate-co-3-hydroxyhexanoate). Macromolecules 28, 4822–4828.
García-Pérez, T., López, J.C., Passos, F., Lebrero, R., Revah, S., Muñoz, R., 2018. Optimization of CH4 removal from diluted emissions and continuous PHB production by methylocystis hirsuta: Towards GHG biorefineries. Chem. Eng. Trans. 68, 439–444.
Khosravi-Darani, K., Mokhtari, Z.B., Amai, T., Tanaka, K., 2013. Microbial production of poly(hydroxybutyrate) from C1 carbon sources. Appl. Microbiol. Biotechnol. 97, 1407–1424.
Koller, M., Salerno, A., Dias, M., Reiterer, A., Braunegg, G., 2010. Modern biotechnological polymer synthesis: A review. Food Technol. Biotechnol. 48, 255–269.
Manjrekar, O.N., Dudukovic, M.P., 2019. Identification of flow regime in a bubble column reactor with a combination of optical probe data and machine learning technique. Chem. Eng. Sci. X 2, 100023.
Myung, J., Galega, W.M., Van Nostrand, J.D., Yuan, T., Zhou, J., Criddle, C.S., 2015. Long-term cultivation of a stable Methylocystis-dominated methanotrophic enrichment enabling tailored production of poly(3-hydroxybutyrate-co-3-hydroxyvalerate). Bioresour. Technol. 198, 811–818.
Padermshoke, A., Katsumoto, Y., Sato, H., Ekgasit, S., Noda, I., Ozaki, Y., 2005. Melting behavior of poly(3-hydroxybutyrate) investigated by two-dimensional infrared correlation spectroscopy. Spectrochim. Acta - Part A Mol. Biomol. Spectrosc. 61, 541–550.
Reilly, I.G., Scott, D.S., Debruijn, T.J.W., Macintyre, D., 1994. The role of gas phase momentum in determining gas holdup and hydrodynamic flow regimes in bubble column operations. Can. J. Chem. Eng. 72, 3–12.
Rikmanis, M., Berzinš, A., Viesturs, U., 2007. Excess turbulence as a cause of turbohypobiosis in cultivation of microorganisms. Cent. Eur. J. Biol. 2, 481–501.
Rodríguez, Y., Firmino, P.I.M., Pérez, V., Lebrero, R., Muñoz, R., 2020. Biogas valorization via continuous polyhydroxybutyrate production by Methylocystis hirsuta in a bubble column bioreactor. Waste Manag. 113, 395–403.
Rostkowski, K.H., Pfluger, A.R., Criddle, C.S., 2013. Stoichiometry and kinetics of the PHB-producing Type II methanotrophs Methylosinus trichosporium OB3b and Methylocystis parvus OBBP. Bioresour. Technol. 132, 71–77.
Ruzicka, M.C., Drahoš, J., Fialová, M., Thomas, N.H., 2001. Effect of bubble column dimensions on flow regime transition. Chem. Eng. Sci. 56, 6117–6124.
Toma, M.K., Systems, F., 1991. Inhibition of microbial growth and metabolism by excess turbulence 38, 552–556.
Urseanu, M.I., 2000. Scaling up bubble column reactors. University of Amsterdam.