

Influence of Aeration on Biomass and Polyhydroxyalkanoate Accumulation in *Priestia flexa* JP1: A Foundation for Nano-Biomaterial Development

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Abstract

Polyhydroxyalkanoates (PHAs) are microbial polymers that have attracted substantial interest in recent times due to their use as biodegradable substitutes for petroleum-derived plastics and also as precursors for nano-enabled biomaterials. The aim of the present work was to evaluate the effect of static and shaking culture conditions on growth kinetics, biomass formation, and PHA synthesis by Priestia flexa JP1. The bacterial strain was cultured in mineral salt media (MSM) at 37 ± 0.2°C under static and shaking culture conditions (120 rpm) for 24-hour intervals. PHA was isolated using a conventional solvent extraction procedure and was weighed. Functional groups of the isolated polymer were analyzed using FTIR spectroscopy. Results showed that agitation led to enhanced PHA synthesis (0.47 g/L) when compared with static cultures (0.42 g/L), while the reverse was observed with biomass formation where static cultures yielded more biomass (0.65 g/L) than shaking cultures (0.57 g/L). Growth kinetics revealed better exponential growth under shaking condition showing increased oxygen transfer efficiency and increased metabolism. FTIR spectroscopy confirmed the presence of characteristic functional groups of PHA which include ester carbonyl group (C=O), C–O–C stretching, and aliphatic C–H bonds under both static and agitation conditions.

Keywords: *Priestia flexa* JP1, PHA, growth kinetics, FTIR, static, agitation

INTRODUCTION

Plastic waste keeps accumulating in environment enforcing researchers everywhere to search for alternative materials that can replace conventional synthetic polymers without harming environment. Polyhydroxyalkanoates (PHAs) have emerged as one of the most important biopolymers as a substitute of synthetic polymers. These are polyesters that bacteria make and store inside their own cells,

essentially as an energy reserve when carbon is in excess amount while keeping other nutrients present in limited quantity. Things that make PHAs attractive is not just that they are biodegradable, biocompatible and behave like thermoplastics but also for their applications in tissue scaffolds, drug delivery systems and other biomedical and nano-biotech applications [1].

However, the actual yield of PHA produced will depend to a large extent on the conditions, such as nutrient balance, oxygen concentration, temperature, pH, as well as whether the culture is stirred or not, affect the result and in many cases, oxygen turns out to be the crucial factor that can redirect the metabolism of bacteria for the production of PHA. If the culture is stirred, then the oxygen will penetrate better into the medium and

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the nutrients will be uniformly distributed, whereas in the static cultures there will be a limited supply of oxygen, which may make the cells switch their metabolic pathways and alter the biomass and polymer accumulation [2]. Such correlation has been established in various studies in relation to aeration: agitation and oxygen concentrations affect hydrodynamic and oxidative stress in bacterial cultures and thus metabolic pathways and final products of cultivation [3]. The effect of oxygen limitation on shifting the metabolism towards accumulation of polymers has been proven in the case of PHA producers. Thus, the study of *Halomonas campisalis* demonstrated how the changes in the parameters of aeration, agitation, and oxygen concentration during the scale-up in fermenter influenced the PHA production [4].

Priestia, a relatively new classification of bacteria that was separated from various *Bacillus* species, has been increasingly studied as an alternative PHA producer. The main advantage of Gram-positive bacterial strains in producing PHA is the absence of the coproduction of lipopolysaccharides, which are considered endotoxins and make the subsequent purification difficult for biomedical application [5]. *Priestia* species have demonstrated great adaptability and can thrive in different environmental conditions ranging from soil and marine sediments to mangrove ecosystems. Particularly, *Priestia megaterium* has been recognized earlier while as a PHA-producing organism capable of growing on various substrates that can be cheap and non-conventional [6].

To be more specific, *Priestia flexa* was previously recognized as an efficient producer of poly(3-hydroxybutyrate) in several isolates from mangrove and halophytic soils, suggesting high potential to produce PHA under various environmental conditions [7]. *Priestia flexa* JP1, a soil isolate from oil-polluted soil that was discovered via 16S rRNA sequencing, is one of these promising bacterial strains [8]. Still, not many studies have been conducted on the response of *Priestia flexa* JP1 to different cultivation conditions.

Recent literature has shown more evidence for the significance of *Priestia* as a genus of PHA producers and the considerable effects of strain and culture conditions on PHA synthesis. The authors compared 12 publicly available *Priestia megaterium* strains cultured with glycerol as the carbon source and found great differences among strains, as they produced 4–42% PHAs with 0.1–1.9 g/L of PHA titer. Several pieces of Research show that a Gram-positive *P. megaterium* strain had the capability to utilize sugarcane molasses and corn steep liquor as inexpensive carbon sources and could produce up to 9.84 g/L of PHA [6–10]. Recently, researchers presented the production of PHB by different *P. megaterium* strains on agro-waste hydrolysates, where the most efficient strain achieved 74.2% of PHB content in optimal glucose culture conditions [11–15].

The importance of oxygen transfer is increasingly being appreciated as another process parameter influencing the PHA productivity. Scientists found that increased levels of dissolved oxygen during the logarithmic phase hastened biosynthesis but caused decreased PHBV formation, thus showing that high rates of growth and maximum polymer formation may occur under different oxygen conditions [16–18]. Similarly, other experiment demonstrated that P3HB formation varied among strains depending on agitation and attributed such findings to oxygen-transfer capability [19, 20]. Together with previous studies concerning the influence of aeration and dissolved oxygen on the growth of PHA-producing cultures [4, 14, 15], such findings justify the current study comparing static and agitated cultivation methods, although it should be kept in mind that the optimal level of agitation/aeration may vary from one strain to another.

This study investigated the behavior of the bacteria *Priestia flexa* JP1 when grown statically compared to agitation regarding growth kinetics, biomass yield and PHA production. Afterward, FTIR analysis performed to confirm the existence of the extracted material and the consistency of its structure in both cultivation modes. This information can then contribute to find the best method of optimizing PHA production in bacteria.

MATERIALS AND METHODS

Microorganism and Maintenance

Priestia flexa JP1, a strain isolated and characterized in earlier work [8] was used throughout this study. Stock cultures were maintained on nutrient agar petri plates and stored at 4°C for further experimental use.

Media Preparation and Growth Condition

Mineral Salt Medium (MSM) was used to culture the bacteria for the production of PHA that contains glucose 20 g/L, peptone 2.5 g/L, yeast extract 2.5 g/L, Magnesium sulphate 0.2 g/L, sodium chloride 0.1 g/L, potassium dihydrogen phosphate 0.5 g/L and pH was maintained 7.0 ± 0.2 [9]. Experiments were performed in triplicates. Culture was revived in nutrient broth medium and after revival 1mL of culture was inoculated in all the flasks in containing MSM medium. Agitated condition flasks were kept in $37 \pm 0.2^\circ\text{C}$ at 120 rpm for bacterial growth while static conditions flasks were kept at $37 \pm 0.2^\circ\text{C}$.

Determination of Growth Kinetics

Samples were collected on daily basis for the assessment of bacterial growth. The optical density of bacteria culture was read using wavelength 600 nm to assess the kinetics of growth of *Priestia flexa* JP1, which is represented as specific growth rate (μ) in d^{-1} using Equation 1 and doubling time using Equation 2 written below [10].

$$\mu = \frac{\ln N_t - \ln N_0}{t - t_0} \quad (1)$$

N_t and N_0 represents number of bacterial cells/mL and μ represents specific growth rate of bacterial cells.

$$t_d = \frac{0.693}{\mu} \quad (2)$$

PHA Extraction from Bacterial Cells

The process of PHA extraction from bacteria was initiated by extracting bacterial biomass in the form of pellet by centrifuging the culture at 7000 rpm for 20 minutes, followed by air drying the pellet before proceeding further. After drying, the pellet was digested in 10 mL of 4% (v/v) sodium hypochlorite at 55°C for 2 hours to digest the bacterial cell wall. This process was followed by washing of digested material using acetone, methanol, and distilled water sequentially to remove the pigment and lipid.

The washed pellet was then dissolved in boiling chloroform, which selectively dissolves the PHA into solution while leaving behind whatever insoluble cell material still remains. That chloroform solution was left at room temperature to evaporate, leaving the purified polymer ready for further analysis.

Finally, the amount of PHA the cells had accumulated was calculated as a percentage by using Equation 3 mentioned below [12].

$$\text{PHA accumulation \%} = \frac{\text{Dry weight of extracted PHA } \left(\frac{\text{g}}{\text{L}}\right)}{\text{Dry cell weight } \left(\frac{\text{g}}{\text{L}}\right)} \times 100 \quad (3)$$

FTIR Analysis

For the characterization of extracted polymer FTIR was done. The dried polymer samples were scanned directly, with no additional derivatization or sample preparation beyond drying, across a spectral range of $4000\text{--}400\text{ cm}^{-1}$ on Perkin Elmer SPECTRUM BX II FT-IR system (USA). The resulting spectra of both cultivation conditions were then examined for the characteristic absorption bands known to correspond to the functional groups found in PHA, confirming the chemical structure of the extracted polymer [13].

RESULTS

Determination of Growth Kinetics

The growth curves of *Priestia flexa* JP1 followed the pattern typical of a bacterial batch culture a short lag phase, a climb through exponential growth, a peak, and then a gradual decline but the two cultivation conditions clearly diverged in how that pattern played out. Both cultures behaved similarly during the initial lag period, but agitation quickly pulled ahead once exponential growth began and that advantage held steady through the peak of growth before both cultures eventually declined and converged again toward the end of incubation can be seen in Figure 1.

This was reflected numerically in the specific growth rate and doubling time as well agitated cultures grew markedly faster and doubled in roughly half the time compared to static cultures (Table 1). Taken together, these results suggest that agitation improves oxygen transfer into the medium, relieving the diffusion limitations that static cultures typically face and allowing cells to divide more efficiently during the early and exponential growth stages. The fact that static cultures eventually approached comparable cell densities, despite growing more slowly, indicates that *P. flexa* JP1 can tolerate oxygen-limited conditions.

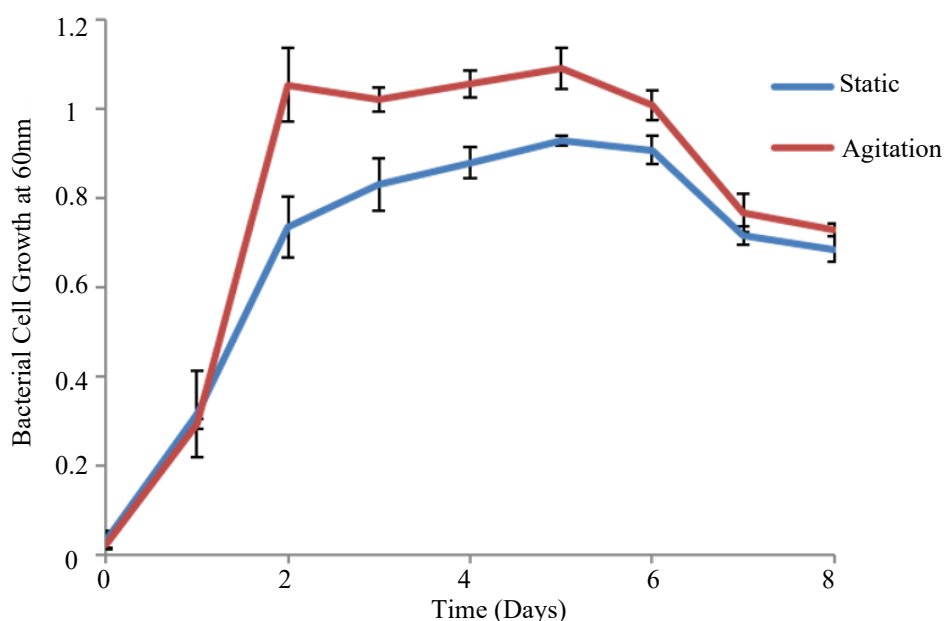


Figure 1. Bacterial cell growth rate in static and agitated culture condition using *Priestia flexa* JP1.

Table 1. Effect of culture cultivation on specific growth rate (μ) and doubling time (t_d) of *Priestia flexa* JP1.

Condition	Specific growth rate (d^{-1})	Doubling time (d)
Static	0.46	1.47
Agitation	0.85	0.81

Production and Extraction of PHA From Bacterial Cell

Comparing the dry cell biomass and PHA yield of these two culture conditions, a trade-off effect became apparent (Figure 2). Static culture had higher cell dry biomass (0.65 g/L) than agitated conditions and on the contrary to that PHA yield was higher in agitated conditions (0.47 g/L) PHA. Moreover, the difference between static and agitated cultures is also obvious when looking at the PHA content in terms of its accumulation percentage (Table 2). Static cultures accumulated PHA in a moderate amount, whereas agitated cultures produced a significantly higher percentage of PHA in spite of the fact that they had less biomass in terms of g/L.

Overall, this suggests that static and agitated cultures cause *Priestia flexa* JP1 to produce different results metabolically. Indeed, under static cultivation conditions, when oxygen is not available as efficiently as under agitation, cells tend to allocate more carbon into biomass rather than to storage. At the same time, under agitation conditions, when oxygen transfer is much faster, cells tend to produce more PHA using their carbon, although the total amount of biomass was low. Oxygen-dependent allocation of carbon resources between biomass and storage is a common trait in PHA-producing bacteria, which shows that oxygen plays the role of a metabolic switch between these two states of matter. This divergence points to a shift in how carbon is allocated between growth and storage depending on oxygen availability cells under agitation appear to channel a larger fraction of their carbon flux into polymer synthesis rather than structural biomass [14, 15].

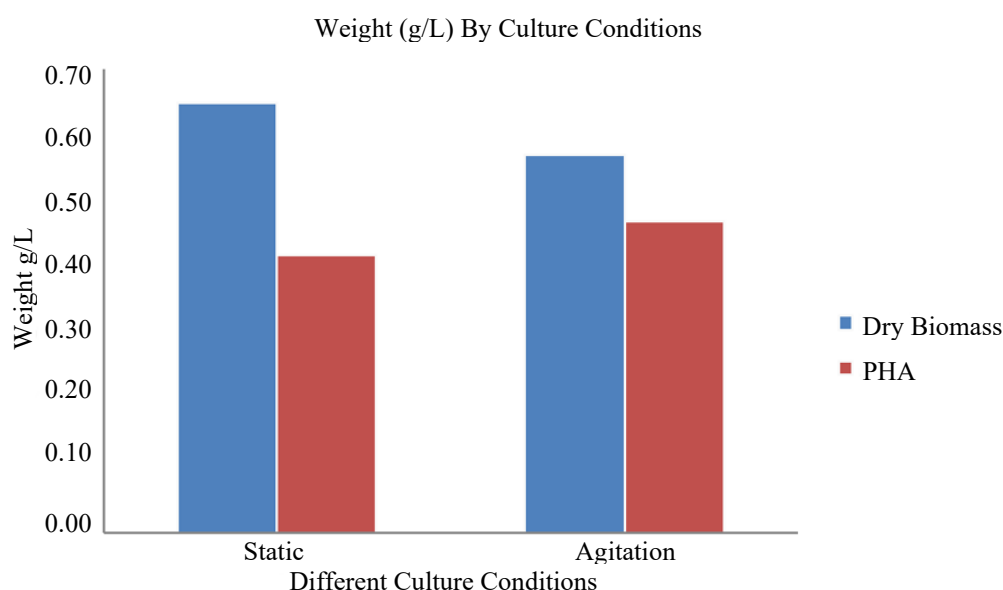


Figure 2. Comparison of dry biomass and PHA extracted in different culture conditions using isolate *Priestia flexa*.

Table 2. Comparative PHA extraction (%) under static and agitated cultivation of *P. flexa* JP1.

Condition	PHA extraction %
Static	64.6
Agitation	82.45

FTIR Spectral Analysis

FTIR spectra confirmed that PHA was successfully produced under both cultivation conditions. Both sample sets displayed the characteristic absorption bands expected of a PHA polyester: a strong ester carbonyl (C=O) stretch near 1720 cm^{-1} , aliphatic C–H stretching between 2850 and 2970 cm^{-1} , CH_2 bending around 1450 cm^{-1} , C–O stretching of the ester linkage between 1270 and 1300 cm^{-1} , and C–O–C stretching vibrations in the 1050 – 1180 cm^{-1} region (Figure 3) [16]. Notably, the spectral profiles of the static and agitated samples were nearly indistinguishable from one another, indicating that while cultivation mode clearly affects how much PHA accumulates, it does not alter the underlying chemical structure of the polymer itself [17–22]. Absorption peaks were detected by FTIR analysis for the isolated PHA, which include absorption due to C–H stretching, ester and carbonyl stretching, $\text{CH}(\text{CH}_2)$ bending, and C–O–C stretching, all of which are consistent with the spectra of other PHAs. The peak at 1720 cm^{-1} due to the ester carbonyl is very close to the peaks for other PHA producing bacteria including the genus *Priestia* [18]. The functional group assignments for the isolated PHA polymer match known PHA structures (Table 3).

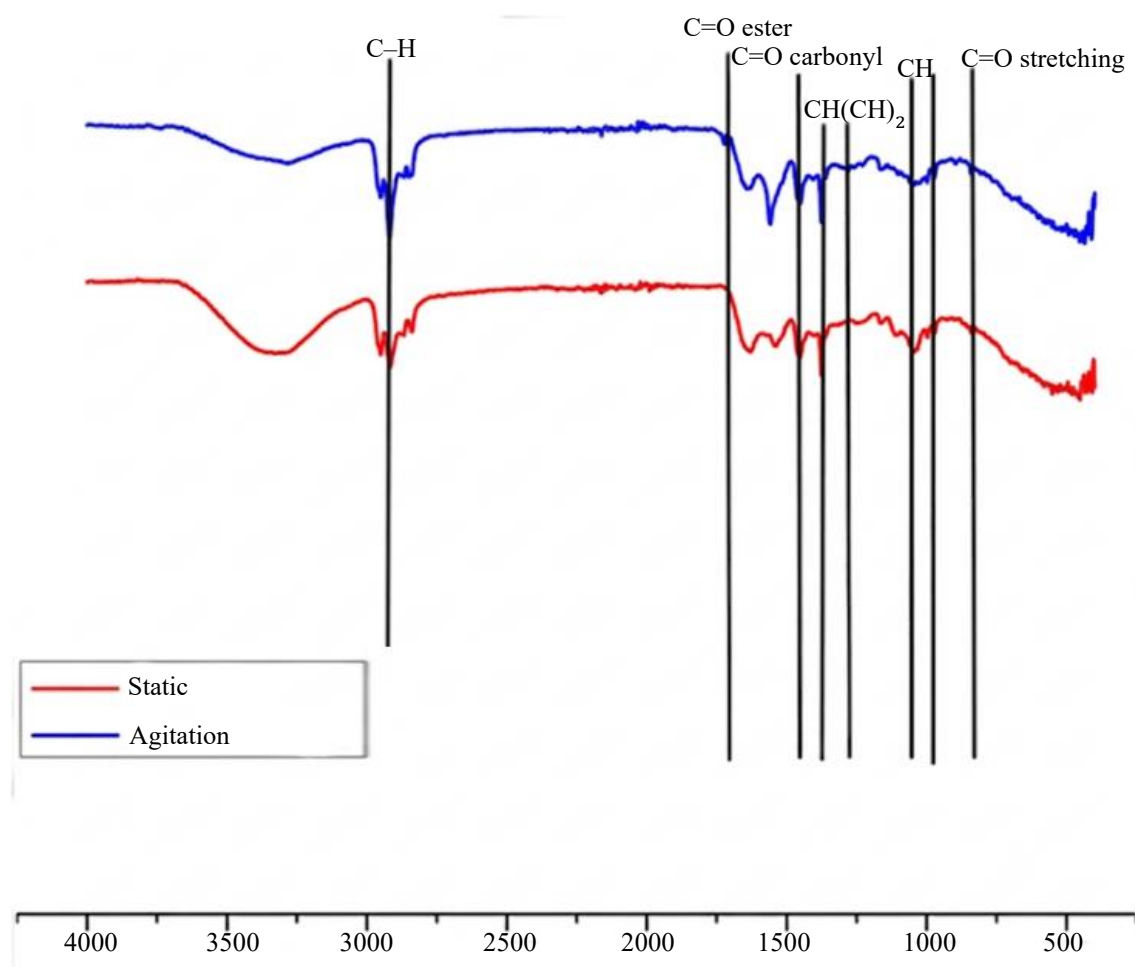


Figure 3. Comparative Fourier transform infrared (FTIR) analysis of static and agitated culture condition using *Priestia flexa* JP1.

Table 3. FTIR functional group assignments for PHA extracted under static and agitated culture conditions of *Priestia flexa* JP1.

Functional group	Wavenumber (cm ⁻¹)	Vibration type	Structural significance
C-H	~2900–2970	Asymmetric/symmetric stretching	Aliphatic side-chain methyl and methylene groups characteristic of the PHA backbone.
C=O (ester)	~1720–1740	Carbonyl stretching	Diagnostic ester linkage confirming the polyester nature of PHA.
C=O (carbonyl)	~1720–1745	Carbonyl stretching	Overlaps with ester band; confirms presence of carbonyl group central to PHA monomer units.
CH(CH ₂)	~1370–1450	Bending vibration	Methyl and methylene bending, reflecting the aliphatic backbone structure.
CH	~1050–1180	C–O–C stretching	Ether-like linkage within the polyester chain, supporting ester bond confirmation.
C=O stretching	~650–950	Skeletal/out-of-plane stretching	Additional confirmation of ester carbonyl environment and polymer backbone integrity.

DISCUSSION

The present study clearly shows that there is a strong correlation between cultivation mode and not only cell growth but also PHA biosynthesis in *Priestia flexa* JP1. Cells growing in agitated culture had higher OD₆₀₀, faster growth and shorter doubling time compared to those grown in static cultivation.

This is due to the better oxygen transfer in shaken cultivation, resulting in improved aerobic metabolism and more effective cell growth [2, 9]. While the amount of total biomass produced in static culture was higher, but it contained less amount of PHA. On the other hand, agitated cultures had more PHA in their cells although low biomass was formed during growth. This shows that the amount of carbon channelled to cell growth and storage is different when the oxygen concentration is different [8]. While in static cultivation more carbon goes to the biomass formation, in agitated condition more of it goes to PHA biosynthesis. FTIR spectroscopy analysis concluded that the polymers produced in either condition have the same functional groups. This means that the cultivation mode affects not the chemical structure of the polymer, but its amount. In other words, it is possible to optimize the yield of the biopolymer while preserving its quality.

Overall, it is clear that the studied strain is metabolically flexible enough to produce more PHA under particular cultivation conditions. Thus, it is possible to increase production of the biopolymer without complicated and expensive changes in cultivation or even genetic modification of the organism [19–24].

CONCLUSION

In summary, the current study illustrated the influence of cultivation mode on growth kinetics, biomass formation and PHA production by *Priestia flexa* JP1. Growth in agitated mode (120 rpm) had a higher specific growth rate (0.85 d^{-1}), lower doubling time (0.81 d), and a higher PHA yield (0.47 g/L) but lower dry biomass formation (0.57 g/L) than that in static mode, while static cultivation had a greater dry biomass (0.65 g/L) than agitated mode. The PHA formation in agitated mode was 82.45%, while it was 64.6% in static mode. FTIR results indicated similar characteristic functional groups of PHA in both modes.

From this study, it can be concluded that agitation is an important cultivation parameter for increasing the PHA production by *P. flexa* JP1 in this bench-scale system, but this does not mean that the strain is industrially productive or economically feasible for any particular nano-biomaterial production. The industrial potential of the strain is suggested as a future research direction. What is needed now is optimization of agitation, aeration/dissolved oxygen, carbon–nitrogen ratio, concentration of substrate, cultivation period, and downstream recovery, which would need to be done via bioreactor studies.

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Ethical Compliance

This manuscript does not report any studies conducted with human participants involving any of the authors.

Conflict of Interest

The authors declare that they have no known financial conflict of interest or any personal relationship that might have appeared to influence the work reported in this paper.

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