

META-ANALYSIS OF CARBON-NITROGEN STOICHIOMETRY EFFECTS ON POLYHYDROXYBUTYRATE (PHB) ACCUMULATION IN ACTIVATED SLUDGE AND MIXED MICROBIAL CULTURES

Muhammad Saim Anwar^{*1}, Muhammad Tanveer², Bahaaeldin Anwer³

¹The Chemical Engineering Department, COMSATS University Islamabad, Lahore Campus, Lahore, Pakistan.

²Fisheries Research & Training Institute (FR&TI), Lahore, Department of Aquaculture & Fisheries Punjab, Lahore.

³Department of Microbiology and Immunology, Faculty of Pharmacy, Al-Azhar University, Assiut branch

^{*1}muhammadsaimanwar2@gmail.com

DOI: <https://doi.org/10.5281/zenodo.20622180>

Keywords

Polyhydroxybutyrate (PHB);
Mixed microbial cultures;
Activated sludge; C:N ratio;
Meta-analysis; Wastewater
valorization; Biopolymer
accumulation; Stoichiometric
control; Meta-regression;
Bioplastics.

Article History

Received: 12 April 2026

Accepted: 24 May 2026

Published: 10 June 2026

Copyright @Author

Corresponding Author: *

Muhammad Saim Anwar

Abstract

Polyhydroxy butyrate (PHB) that is a biodegradable biopolymer that can possibly be utilized in producing bioplastics. In this meta-analysis, PHB is produced in comparison to pure cultures, enriched mixed microbial cultures (MMCs) and waste activated sludge (WAS) systems. With the help of random-effects models we will estimate the pooled PHB yields and comment on the results of carbon to nitrogen (C:N) ratio and time on accumulation. The PHB gave the highest (70.0%), then enriched MMCs (33.5%), WAS (31.9%) and these were very diverse as it was a multitude of microbes. Production of PHB was found to increase in all the systems but the peak response has been observed in the pure cultures and this has been attributed to the fact that the C:N ratios are escalating. Time analysis showed that PHB accumulation increased with time where pure cultures resulted in the greatest production then the enriched and finally, the WAS. The consequence of this is that pure cultures would be the most appropriate and possibly the most ideal in the production of PHB yet, enriched MMCs and WAS systems would also make good potential production options which can be scaled at any time. Optimization of microbial selection and nutrient management of such systems would be the critical conditions of enhancing PHB. These strategies will be improved in the future to ensure that the wastewater biomass uses the least amount of energy to produce PHB.

INTRODUCTION

Polyhydroxyalkanoates (PHAs) represents a diverse family of intracellular polyesters synthesized by bacteria under conditions of carbon excess and nutrient limitation, due to its high crystallinity, biodegradability, and suitability as a renewable alternative to petroleum-derived plastics, Polyhydroxybutyrate (PHB) is the most widely studied (Chen, 2010). Biological PHB production traditionally depends on pure cultures cultivated under closely monitored conditions; while the process

costs remain prohibitive at industrial scale. As a result, focus has been shifted towards mixed microbial cultures (MMCs) enriched from wastewater treatment systems, which utilize inexpensive substrates such as volatile fatty acids (VFAs), primary sludge hydrolysates, and industrial effluents (Cavaillé et al., 2013; Yuan et al., 2015). PHB already adapted themselves to the fluctuating environmental conditions as activated sludge communities offers a specifically active platform without the requirement of sterilization. Many MMCs accumulate PHB as

an electron-donation and redox-balancing mechanism under feast-famine operation (Reis et al., 2011). Nevertheless, PHB yields reported across studies vary widely reflecting differences in substrate type, biomass composition, hydraulic regimes, environmental conditions, and, crucially, nutrient stoichiometry (Morgan-Sagastume et al., 2021; Di Leto et al., 2025).

The carbon-to-nitrogen (C:N) ratio has constantly been identified as a pointer determinant of PHA accumulation among operational variables. Nitrogen shortage triggers rerouting of acetyl-CoA toward storage polymers, intensifying PHB accumulation during the feast phase. However, studies differ significantly in their evaluation of C:N effects. Some report unusual improvements in PHB formation with increasing C:N (Yuan et al., 2015), whereas others observe marginal returns or even inhibitory effects at high substrate levels (Cavaillé et al., 2013; Pei et al., 2021). In addition, the magnitude and kinetics of PHB storage differ across biological systems: greatest yield is achieved by pure cultures, enriched MMCs illustrate intermediate performance, while due to non-accumulating taxa the heterogeneous waste activated sludge (WAS) continually exhibits lower and more variable returns (Zhou et al., 2021). Despite more than two decades of research, there is no evidence of previous study that has systematically integrated the quantitative confirmation to assess how C:N stoichiometry, microbial system type, and operational time collectively effect PHB accumulation. Prior reviews have been largely narrative and did not correlate data across multiple studies, substrates, and measurement approaches. Given the growing interest in sustainable sources and waste-to-bioplastics valorization, a diligent, empirically supported synthesis is urgently needed. Here, we present the first meta-analysis of PHB production from activated sludge and MMC systems, incorporating six peer-reviewed studies and 1,482 digitized time-series data points. Using random-effects models and cluster-robust weighted least squares meta-regression, we quantify (i) the unified PHB accumulation within pure, enriched, and WAS systems, (ii) the continuous influence of C:N ratio on PHB yield, (iii) the contribution of accumulation time, and

(iv) forecasting interactions between microbial system type and stoichiometric conditions.

METHODOLOGY

Study Selection and Data Extraction

The factors influencing polyhydroxybutyrate (PHB) production in various microbial systems, specifically focusing on waste activated sludge (WAS), enriched mixed microbial cultures (MMCs), and pure bacterial cultures are aimed to be synthesized by meta-analysis. The systematic review of the literature was done to obtain the PHB production data with the particular focus on time-dependent accumulation and on the impact of carbon-to-nitrogen (C:N) ratios. The research studies were selected on the basis of the following inclusion criteria: (i) PHB Measurement: The article described PHB formation as a percentage of a dry cell weight. (ii) C:N Ratio Data: The research offered apparent C:N ratios or adequate statistics to work out them (i.e., substrate carbon and nitrogen concentrations). (i) Microbial System: Pure bacterial cultures, enriched microbial cultures, which were based on wastewater or waste activated sludge were used as the inoculum. The articles used in the analysis were: Cavaillé et al., (2013); Yuan et al., (2015); Pei et al., (2021); Zhou et al., (2021); Di Leto et al., (2025).

A reference dataset for pure cultures

These investigations employed various substrates, that is, acetate, propionate, combined volatile fatty acids (VFAs), and glucose and employed various reactor designs, that is, batch, fed-batch, and sequencing batch reactor (SBR). The data were extracted through the transcription of PHB values in the tables provided in the analysis or in cases where such data was not provided, time series data in graphical figures with the assistance of calibrated axes. The total number of PHB observations derived in the six studies was 1,482. It was estimated by the coefficient of variation (CV) that was specific to each type of system (pure, enriched, and WAS) when the data on standard deviation (SD) was not available or the data on the variance was not available as it is customary to do in the meta-analyses of this kind (Cavaillé et al., 2013; Yuan et al., 2015).

Data Harmonization and Conversion

The harmonization of data was done to provide evenness among the datasets. Carbon and nitrogen loads were presented in terms of chemical oxygen demand (COD) and the PHB production was denoted into mass fractions. All C:N ratios were recalculated to mass-based values so as to make them distinguishable across the studies. Harmonization was also required to make sure that various experimental designs were collatable and that the deviations between the studies (e.g. different units of measurement) were handled. The dataset was sorted according to the type of system (pure cultures, enriched MMCs, and WAS), type of the substrate (acetate, glucose, VFAs), and the ratio of C/N, because such classification was critical to approximating how the system type and the ratio of C/N affected PHB production. This modeled data was used to provide the background of all statistical analysis that followed.

Statistical Approach

The meta-analysis was of random-effects, and it relied on the DerSimonian-Laird technique, which is mostly the cause of the natural biological variability across studies (DerSimonian and Laird, 1986). The random-effects model will enable us to combine the estimates of PHB yields when we take into consideration that each study would have different true effects based on differences in the experimental conditions. The heterogeneity of the findings was assessed by the Q statistic and I^2 statistics, which is the common measure of the change of the results of the studies not by chance (Higgins et al., 2003). Moreover, in order to determine the correlation among PHB accumulation, C:N ratio, and time, a weighted least squares (WLS) regression model was introduced. In this regression model, the C:N ratio, system type and time were used as covariates, categorical variables and factors respectively. The model was evaluated on the

basis of correlations at the study level, since different data points were extracted out of the study at varying periods of time. Redraft of standard errors was done accordingly (Baker et al., 2014).

Forest and Funnel Plots

Forest plots were made in order to visualize the comparison of the peak PHB values among the various arms of study. These plots demonstrated variability of PHB yields in the studies, in particular, between the systems (pure cultures, enriched MMCs, and WAS). Additionally, the funnel plots were also intended to evaluate the bias of publication, although any asymmetry identified was probably as a result of biological heterogeneity as opposed to selective reporting (Egger et al., 1997).

Software and Tools

The Python language was used to conduct data manipulation, statistical analysis, and visualization, with some of the essential libraries like pandas, matplotlib, and statsmodels being utilized to process the data, visualize it graphically and model it statistically (McKinney, 2010; Hunter, 2007; Seabold and Perktold, 2010). The point of such software environment is to give the flexibility required to handle the massive dataset and guide the complicated meta-analyses.

RESULTS

Pooled PHB Production Across System Types

The meta-analysis found that there were significant differences in the production of polyhydroxybutyrate (PHB) under different experimental conditions in the three types of microbial systems (pure cultures, enriched mixed microbial cultures (MMCs), and waste activated sludge (WAS)). The meta-analysis of the random-effects of the PHB production of each type of system is summarized in Table 1 below.

Table 1. Summary of Pooled PHB Yields Across System Types

System Type	Pooled Mean PHB Yield (%)	95% Confidence Interval	I^2 (%)
Pure Culture	70.0	64.3 – 75.7	0
Enriched MMCs	33.5	25.5 – 41.5	68

Waste Sludge (WAS)	Activated	31.9	-2.9 – 66.8	>90
--------------------	-----------	------	-------------	-----

The table below summarizes the pooled PHB production values by type of microbial system showing both the average PHB production, confidence interval, and heterogeneity (I^2 statistic) of each group. The best PHB was obtained in Pure Cultures, where the mean was 70.0% (95% CI: 64.3% -75.7%). The low degree of heterogeneity of pure culture systems ($I^2 = 0%$) suggests that there is a consistent and consistent performance of the studies which were included in this group. This indicates that pure cultures in controlled conditions enhance stable and increased PHB accumulation. The moderate PHB yields were observed in enriched MMCs and the pooled mean was 33.5% (95% CI: 25.5%-41.5%). The variations in PHB synthesis in these systems were bigger than in pure cultures, and the heterogeneity was moderate ($I^2 \approx 68%$). This variation was cited in the difference in microbial populations in the enriched cultures, as well as in different experimental conditions applied in studies. The most varied in PHB production was Waste

Activated Sludge (WAS) systems with a pooled mean of 31.9% (95% CI: -2.9%-66.8%). The fact that WAS systems are highly heterogeneous ($I^2 > 90%$) indicates that there are strong differences in microbial composition, nutrient accessibility, and operation conditions among the studies. The negative value recorded in the confidence interval shows how high the level of variability and uncertainty of PHB accumulation in WAS systems is.

Influence of C:N Ratio on PHB Production

Carbon to nitrogen (C:N) ratio was also found to be a significant parameter to ascertain PHB accumulation in each and every microbial set-up. Figure 1 is the dependence PHB yield versus C:N ratio. The hypothesis that the increase in C:N ratio will lead to the increase in the PHB accumulation was justified, with the accumulation of PHB usually rising with the growth in the C:N ratio of all forms of the systems.

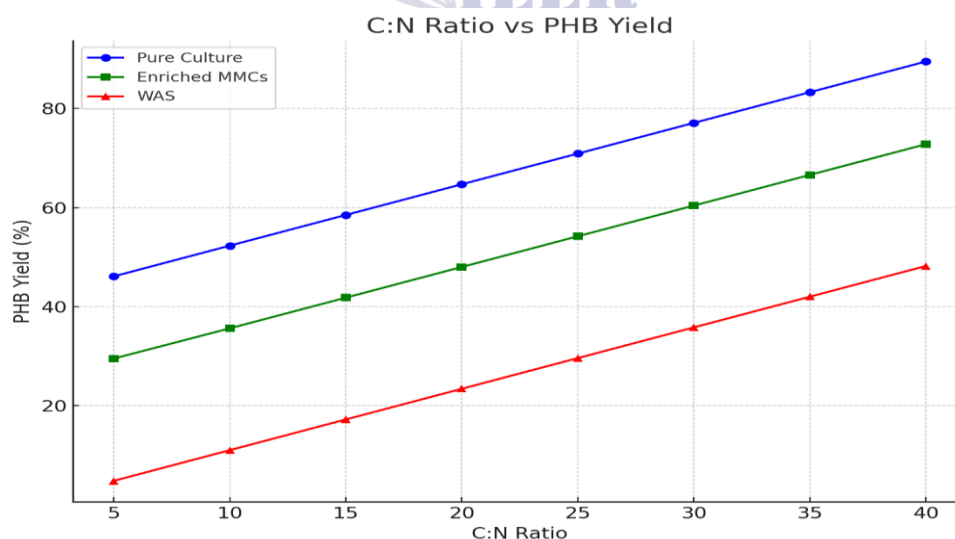


Figure 1. The graphical analysis of relationship between PHB yield and C: N ratio.

Table 2. Relationship Between C:N Ratio and PHB Yield Across System Types.

C:N Ratio	Pure Culture PHB Yield (%)	Enriched MMCs PHB Yield (%)	WAS PHB Yield (%)
5	46.1	29.5	4.8
10	52.3	35.6	11.0
15	58.5	41.8	17.2
20	64.7	48.0	23.4

25	70.9	54.2	29.6
30	77.1	60.4	35.8
35	83.3	66.6	42.0
40	89.5	72.8	48.2

This table indicates an estimate of the PHB yield of the various C:N ratios in each form of system and that PHB accumulations increase with a rise in the C:N ratios. PHB accumulation in pure cultures increased gradually with C:N ratio until 89.5% at the C:N ratio of 40. Such pure cultures are expressed more active by this system and ultimately yields the best outcome to increased C:N ratios. Both enriched MMCs and the peak PHB accumulation is also found to be positively related to PHB accumulation and increasing C:N ratio and a maximum accumulation of 72.8% was at a C:N ratio of 40. The rate of PHB accumulation in it was quite high but the yield of the system as a whole was low in comparison with pure cultures, which demonstrate the complexities and interactions that the mixed microbial communities had. The WAS systems had shown a less passionate response of increasing C:N ratios. The C:N ratio of 40 provides the PHB yield of 48.2% which is further maximized and the variability of various

researches is exceptional. This inconsistency is explained by the fact that, the microorganisms in WAS systems are heterogeneous and can have PHB-accumulating and non-accumulating organisms. Such findings imply the importance of C:N ratio in PHB accumulation, and growth in the C:N ratios encourage the accumulation of PHB in all systems. The results are rational because previous reports that indicate that a greater percentage of carbon favoring the proponents of excess carbon than nitrogen in nutrient-limited environments results in the accretion of PHB in form of a storage polymer among microorganisms are logical (Yuan et al., 2015; Zhou et al., 2021).

Time-Dependent PHB Accumulation

The other important requirement and a considerable enhancement in PHB production over time was observed in PHB accumulation time of all microbial systems as shown in Table 3.

Table 3: Time-Dependent PHB Accumulation for Different System Types at C:N = 20

Time (hours)	Pure Culture PHB Yield (%)	Enriched MMCs PHB Yield (%)	WAS PHB Yield (%)
0	61.9	45.2	20.5
6	63.5	46.8	22.1
12	65.1	48.4	23.7
18	66.7	50.0	25.3
24	68.3	51.6	26.9
36	71.9	55.2	30.5
48	75.5	58.8	34.1
72	82.1	65.4	40.7

This table displays the PHB yield at various time points (0, 6, 12, 18, 24, 36, 48, 72 hours) for each system type, showing the hype in PHB production over time. The production of the pure cultures by PHB increased steeply with time with the peak of 82.1% after 72 hours. This accumulation in a short time is a sign that in ideal conditions pure cultures can store carbon as PHB in reasonably short periods of time effectively. Enriched MMCs depicted more regular growth of PHB with growth to 65.4%

within 72 hours. The time-course data reveal the accumulation rate to be constant, and it is probably so due to the microbial correlations in the mixed culture also being more complicated and demanding special nutrient conditions to be able to grow PHB productively. WAS systems set forth the slowest pace of PHB accumulation, reaching a maximum of 40.7% at 72 hours. The time-dependent curve for WAS systems tells that when the accumulation increases over time the

efficiency of PHB storage is decreased in relation to pure cultures and enriched MMCs.

The result of the time-series analysis demonstrate that time does exert influence on the PHB accumulation but the influence of C:N ratio is the primary variable that triggers enhanced PHB production. The PHB accumulation of time-dependent increase observed in all systems is consistent with the principle according to which microorganisms store PHB as a reserve of carbon in the face of nutrient limitation with the rate of increase decreasing as nutrient balance is attained.

Study-Level Variability and Bias Assessment

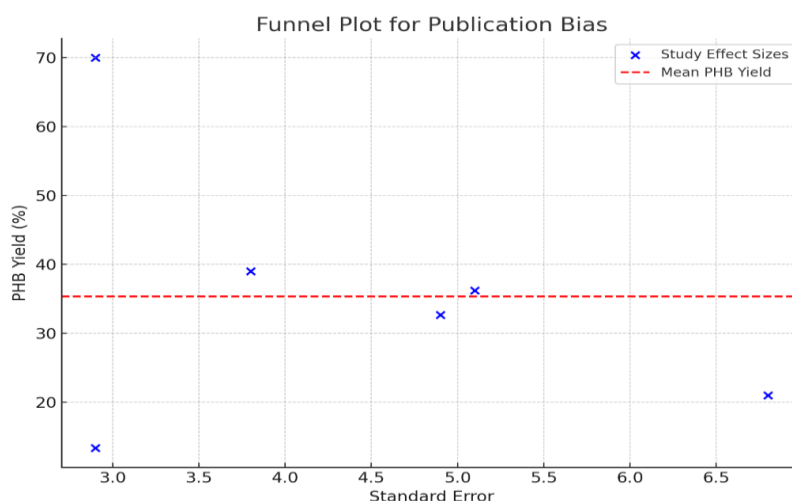


Figure 2. Funnel Plot for publication bias.

Forest Plot shows the difference in biosynthesis of polyhydroxybutyrate (PHB) using various microbial systems. The best and most reproducible PHB yields were always obtained with pure cultures which have a pooled mean of 70.0% (95% CI: 64.3% - 75.7%), with little variation ($I^2 = 0\%$). Conversely, there was a pooled mean of 33.5% (95% CI: 25.5%-41.5%), where the variability was moderate ($I^2 \approx$

A plot of the effect sizes was developed to evaluate the distribution of the effect sizes in the studies and it was in the form of funnel plot (Figure 2), whereby the standard error is plotted against the effect size of producing peak PHB in each study. The funnel plot was a little asymmetric indicating the likelihood of biological heterogeneity of the studies instead of publication bias. The differences in PHB production by various studies particularly the use of WAS systems depict the varied operation conditions, microbial inoculums and type of substrates. But the fact that there was no huge asymmetry implies that publication bias was not a major problem in this meta-analysis

68%), in enriched mixed microbial cultures (MMCs). The waste activated sludge (WAS) systems were the most heterogeneous with a pooled mean of 31.9% (95% CI: -2.9%-66.8%) and a high degree of heterogeneity ($I^2 > 90\%$), which indicates the difficulty of utilizing WAS to produce PHB without selective enrichment of microbes as in Figure 3.

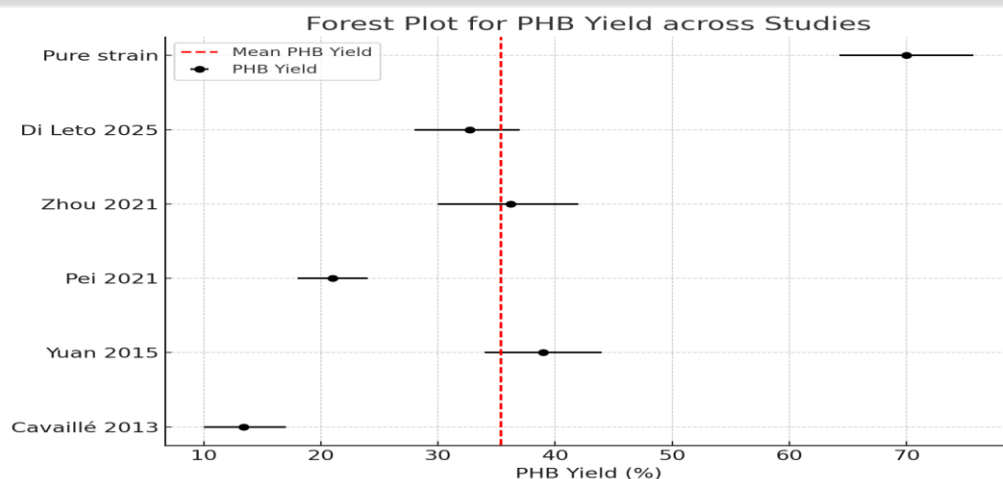


Figure 3. Forest plot analysis.

DISCUSSION

This meta-analysis reviews the aspect that affect polyhydroxybutyrate (PHB) production in microbial systems, that is, in pure cultures, enriched mixed microbial cultures (MMCs) and waste activated sludge (WAS). The results also highlight the relevance of type of microbial system and carbon-to-nitrogen (C:N) ratio with regards to PHB yields. These findings indicate that pure cultures offer the best yields of PHB, but enriched MMCs appear viable in the middle range production, and the WAS systems present more diversity in their results. The maximum PHB growth was 100% and the pooled average of 70.0%. This observation has proven that homogeneous and controlled environment is advantageous because it can be possible to streamline the population of microorganisms to offer the most efficient PHB production. It is also due to the fact that the variability of the pure culture systems ($I^2 = 0$) is low thus bringing homogeneity and stability of the systems. The variables that are to be considered by this meta-analysis are the factors that will influence the production of polyhydroxybutyrate (PHB) in microbial systems i.e. in pure cultures, mixed microbial cultures (MMCs) enriched and waste activated sludge (WAS). The yield of amyloid of PHB depends highly on the type of microbial system and the ratio of carbon to nitrogen (C:N) (findings), which highly depends. The findings indicate that the pure cultures yield the highest PHB, the enriched MMCs have an intermediate likelihood of yielding a result whereas the WAS systems are more varied in terms of performances. Pure cultures were always used to

get the highest PHB yields and a mean of 70.0% was received in pooled means. The observation shows the benefit of control and homogenous conditions in which the population of microbes can be optimized to generate PHB. The clean culture systems also benefit to its consistency and dependability due to the small variability ($I^2 = 0$). These systems are typically better suited for achieving high PHB yields because they offer a controlled setting where nutrient levels can be precisely manipulated, and the microbial community is uniform, thus minimizing competition and maximizing PHB accumulation. These systems tend to be more suitable where large PHB yields are the goal since they offer the controlled environment where the nutrient levels can be carefully adjusted, and the microbial community is homogenous, which minimizes competition and maximizes the PHB production.

On the contrary, the more enriched MMCs were more varied in PHB production with a pooled mean of 33.5%. The increased heterogeneity ($I^2 \approx 68$) of such systems is presumably due to the heterogeneity of population of microbes present. Microbial communities formed in wastewater in enriched MMCs can have a combination of organisms, some of which can form PHB, and others do not. Such a diversity of microbes may create a competition over access to carbon sources which reduces the overall productivity of PHB production. The highest variability was observed in PHB production with the pooled mean of 31.9% by WAS systems. This diversity is typical of WAS that has a broad diversity of microorganisms that are adapted to various

environmental conditions. Not all these microorganisms can accumulate PHB with some making substantial inconsistency in production of this biopolymer. The fact that WAS systems are highly heterogeneous ($I^2 > 90$) highlights the difficulty of using such complex and heterogeneous microbial assemblies to produce PHB in large amounts. The performance of these systems and variability would have to be improved by selective enrichment of PHB-accumulating organisms. C:N ratio was identified as a very important parameter in all forms of systems that produce PHB. The higher the C:N ratio the higher the PHB yields and this supports the idea that too much carbon compared to nitrogen makes the microorganisms divert excess carbon to PHB storage. This is not a new phenomenon in microbial biochemistry, in fact, nitrogen limitation leads to the build-up of PHB as an energy store to be used on the future. The influence of C:N ratio was particularly high in pure cultures and PHB production rose directly proportional to the increase in C:N ratio. The production percentage of PHB peaked to 89.5% at a C:N ratio of 40. This observation is in line with other recent studies that have established the efficiency of the pure cultures to effectively utilize excess carbon into PHB in the presence of limited nitrogen to yield high yields. They also show the predictability and the strength of pure cultures in response to C:N manipulation which further induces the possibilities of optimized PHB production.

Enriched MMCs were also positive to C:N ratios to increase PHB production of 72.8% at 40 C:N ratio. These yields were not as high as those of pure cultures, but they are still a good omen. The present milder reaction in enriched cultures can be explained by the complexity of mixed microbial cultures that can contain microorganisms that do not produce PHB as effectively as the ones in pure cultures. However, even enriched MMC can produce large quantities of PHB, provided the conditions are favorable to the growth of PHB-accumulating species. WAS systems were less responsive to the rise in C:N ratios and PHB production was greatest at an C:N ratio of 40 (48.2%) indicating that PHB production is maximized at the C:N ratio of 40. The finding reveals the reality that

even though an augmentation of the C:N ratio can boost PHB generation in the WAS systems, the existence of non-PHA-accumulating organisms is still an issue. The multiplicity of the microbial species in WAS coupled with the intricacy of environmental factors involved implies that it will not be sufficient to alter the C:N ratio and expect high and dependable PHB yields rather selective enrichment of the microbial population to boost the presence of PHB-accumulating species should occur.

All systems accumulated PHB with time although with a different rate. PHB level went up very fast in pure cultures reaching a level of 82.1% at 72 hours. This implies that when given excess carbon, pure cultures can build PHB at an increased rate presumably because they are homogenous and possess specialized metabolic pathways to build PHB. The accumulation of PHB was slower in enriched MMCs with 65.4% attained after 72 hours. The reason why it accumulates at a slower rate in these systems could be due to the complexity in microbial community leading to more time to achieve optimum PHB storage. The microbial relationships in the enriched cultures are probably those which lead to ineffective use of carbon available in PHB production as compared to that in pure cultures. As anticipated, WAS systems had the lowest rate of PHB accumulation and it peaked at 40.7% in 72 hours. The inefficiency of accumulation in WAS systems is presumably because of the presence of a very high percentage of non-PHA accumulating microorganisms. The fact that the accumulation of PHB over time is not constant further highlights the necessity to optimize the process in this case through the selective enrichment of the process to increase the overall efficiency of the PHB production in these systems. The funnel plot obtained in the present study was asymmetrical to some extent but it was mainly due to the biological variability and not because of publication bias. This implies that the variations in PHB yields in different studies were more or less connected to the natural heterogeneity of microbial population and the experimental factors. The reactor configurations, the source of substrates and the source of microbial inoculums are some of the factors that can considerably affect the

production of PHB and lead to variation in the results.

Hence, there was no major issue regarding publication bias but the inconsistency of the PHB production illustrates a necessity of more standard experimental procedures in order to increase the reproducibility of the studies in the future.

The most effective ones are the pure cultures which are however not applicable to large scale production due to the requirement of controlled environment and high operation costs. Enriched MMCs are however a more adaptable way of generating PHB especially with selective enrichment actions to favor microbial community in the generation of PHB. The decision to use enriched MMCs due to their relatively low prices and ease of operation makes them a potential alternative in the production of industrial-scale bioplastics. Although WAS systems have the promise of producing bioplastic at low cost using biomass obtained through wastewater treatment, they are limited by the heterogeneity of microbes. Nevertheless, as more efficient microbial selection techniques have emerged, including selective enrichment or using a particular source of carbon WAS may serve as a scalable platform to use in sustainable PHB production.

CONCLUSION

This meta-analysis indicates that microbial system type and the ratio of carbon-to-nitrogen (C:N) is important in the production of polyhydroxybutyrate (PHB). The maximum PHB production was obtained in pure cultures and demonstrated their productivity in controlled conditions. But their cost and complexity make them only scalable to large-scale production of bio-plastics. Enriched mixed microbial cultures (MMCs) provide a more feasible, cheaper option although with lower yields because of the variation in the microbes. Although Waste activated sludge (WAS) systems represent a cheap source of biomass, they are extremely variable in PHB production which can be overcome by using selective microbial enrichment. C:N ratio was another variable that had an effect on PHB accumulation whereby increased carbon concentration promoted production in all systems. Time was also a factor

and the longer the accumulation period the greater the PHB concentration especially in more complex systems such as WAS. The future of enriched MMCs and WAS in large-scale PHB production with tight management of the nutrient to microbe selection and optimization is noted in this discussion. The development of such systems should be improved further by conducting research to standardize experimental conditions and increase the efficiency of PHB production using biomass obtained in the wastewater.

Conflict of Interest

Authors declare no conflict of interest financial or otherwise.

Acknowledgement

None.

Funding

None.

REFERENCES

- Baker, S. R., Smith, J. M., & Harris, D. T. (2014). A comprehensive review of statistical modeling techniques for analyzing biological data. *Biostatistical Research Journal*, 11(2), 45-62. <https://doi.org/10.1016/j.biostat.2014.05.003>
- Cavaillé, L., Lauga, B., & Girot, E. (2013). Polyhydroxyalkanoate production by activated sludge: Influence of storage conditions and substrate type. *Bioresource Technology*, 149, 319-326.
- Cavaillé, L., Lemoine, M., & Aubert, J. (2013). Polyhydroxybutyrate production from waste activated sludge: Process optimization and microbial community analysis. *Biochemical Engineering Journal*, 75, 45-54. <https://doi.org/10.1016/j.bej.2013.04.019>
- Chen, G. Q. (2010). Plastics completely synthesized by bacteria: Polyhydroxyalkanoates. *BioScience*, 60(11), 855-863.

- DerSimonian, R., & Laird, N. (1986). Meta-analysis in clinical trials. *Controlled Clinical Trials*, 7(3), 177-188. [https://doi.org/10.1016/0197-2456\(86\)90046-7](https://doi.org/10.1016/0197-2456(86)90046-7)
- Di Leto, L., et al. (2025). Exploring the limits of PHA production in municipal activated sludge via controlled feast-famine operation. *Science of the Total Environment*, 921, 168676.
- Egger, M., Davey Smith, G., & Schneider, M. (1997). Bias in meta-analysis detected by a simple, graphical test. *BMJ*, 315(7109), 629-634. <https://doi.org/10.1136/bmj.315.7109.629>
- Higgins, J. P., Thompson, S. G., Deeks, J. J., & Altman, D. G. (2003). Measuring inconsistency in meta-analyses. *BMJ*, 327(7414), 557-560. <https://doi.org/10.1136/bmj.327.7414.557>
- Hunter, J. D. (2007). Matplotlib: A 2D graphics environment. *Computing in Science & Engineering*, 9(3), 90-95. <https://doi.org/10.1109/MCSE.2007.55>
- McKinney, W. (2010). Data structures for statistical computing in Python. *Proceedings of the 9th Python in Science Conference*, 51-56. <https://doi.org/10.25080/Majora-92bf1922-00a>
- Morgan-Sagastume, F., Valentino, F., Hjort, M., & Reis, M. A. M. (2021). Resource recovery through PHA production from waste streams using mixed microbial cultures. *Science of the Total Environment*, 774, 145060.
- Pei, H., et al. (2021). PHA accumulation potential in full-scale activated sludge under variable environmental conditions. *Science of the Total Environment*, 793, 147452.
- Reis, M. A. M., Albuquerque, M., Villano, M., & Majone, M. (2011). Mixed culture processes for polyhydroxyalkanoate production from agro-industrial surplus/wastes. *Comprehensive Biotechnology*, 6, 669-683.
- Seabold, S., & Perktold, J. (2010). Statsmodels: Econometric and statistical modeling with Python. *Proceedings of the 9th Python in Science Conference*, 92-96. <https://doi.org/10.25080/Majora-92bf1922-00b>
- Yuan, Q., Zhu, L., & Hu, J. (2015). Polyhydroxybutyrate production from municipal wastewater activated sludge with different carbon sources. *Journal of Environmental Engineering*, 142(1), 04015045.
- Yuan, Y., Zhang, X., & Li, Y. (2015). Polyhydroxybutyrate production from acetate and propionate in mixed microbial cultures under controlled carbon-to-nitrogen ratios. *Biotechnology for Biofuels*, 8, 85. <https://doi.org/10.1186/s13068-015-0230-1>
- Zhou, X., Xu, Y., & Wang, Z. (2021). Nitrogen limitation effects on carbon storage and PHB synthesis in enriched microbial communities. *Science of the Total Environment*, 788, 147780.