JEB®

Journal of Environmental Biology

p-ISSN: 0254-8704 • e-ISSN: 2394-0379 • CODEN: JEBIDP

Journal website : www.jeb.co.in ★ **E-mail :** editor@jeb.co.in

Triveni Enterprises (Educational Services Set-up)

Original Research

DOI : http://doi.org/10.22438/jeb/44/4/MRN-5096

Kinetics of biomass and polyhydroxyalkanoates synthesis using sugar industry waste as carbon substrate by *Alcaligenes* sp. NCIM 5085

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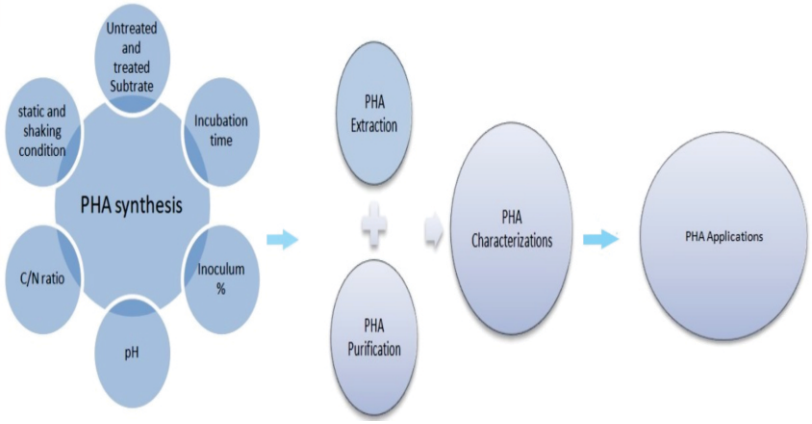
Received: 23.09.2022 *Revised*: 18.10.2022 *Accepted*: 31.12.2022

Abstract

Aim: To optimize the operating parameters and further determine the kinetics of biomass and polyhydroxyalkanoate synthesis using sugar industry waste as a carbon substrate by *Alcaligenes* sp. NCIM 5085.

Methodology: Molasses, clarified and pretreated with sulphuric acid, was taken in a batch reactor for fermentation to generate biomass and further for polyhydroxyalkanoate synthesis where *Alcaligenes* sp. *NCIM 5085* was used as a culture. A number of parameters were observed during fermentation experiments to optimize the biomass and PHA namely: initial substrate concentration, incubation period, C/N ratio, inoculum concentration and pH. Fermented solution was filtered to account the yield of biomass from the solution at each time interval decided for each experiment and polyhydroxyalkanoate was extracted from the biomass by chloroform and methanol extraction. Growth kinetics of biomass was modeled by Monod and Logistic models whereas Luedeking-Piret model was used for polyhydroxyalkanoate production rates.

Results: From the fermentation experiments, it was found that for 40 g l^{-1} of molasses at neutral pH, 4.48 g l^{-1} of biomass was synthesized when fermentation was observed for 48 hr. One percent of cell dry weight of biomass was extracted as polyhydroxyalkanoate. Logistic model for growth kinetics and Luedeking-Piret model for polyhydroxyalkanoate production rate were found in agreement with the experiments where regression coefficient was maximum with minimum error.



Interpretation: The PHA growth kinetics and PHA production kinetics done in this work can be utilized to design a bioreactor for PHA production at industrial scale.

Key words: *Alcaligenes* sp., Biomass, Cane molasses, Growth kinetics, Polyhydroxyalkanoates

How to cite : Kanzariya, R., A. Gautam, S. Parikh and S. Gautam: Kinetics of biomass and polyhydroxyalkanoates synthesis using sugar industry waste as carbon substrate by *Alcaligenes* sp. NCIM 5085. *J. Environ. Biol.*, **44**, 612-622 (2023).

Introduction

Plastics have become an essential aspect in today's modern world. It is the most extensively used material around the globe. Polypropylene, polyethylene and polyethylene terephthalate are conventional plastics, derived from fossil fuels; they are non-biodegradable in nature, causing pollution and hazardous for humans and environment. With the rising concern for a green environment, biodegradable biopolymers like polyhydroxyalkanoates, polysaccharides and polylactic acid synthesized from natural renewable resources has attracted immense attention owing to their biodegradability and environment eco-friendly nature. (Akinwumi *et al.,* 2022; Tania *et al.,* 2022; Guo *et al.,* 2022). Polyhydroxyalkanoates are biodegradable, biocompatible and a class of linear polyester. They are produced by numerous Gram-negative and Gram-positive bacterias during fermentation of carbon sources under nutrient-deficient conditions.

The bacterial strains used in polyhydroxyalkanoate biosynthesis are categorized based on the fermentation medium used. For example: *Cupriavidus necator, Protomonas oleovorans*, and *Extorquens* bacteria require nutrient limitation with surplus carbon sources to accumulate polyhydroxyalkanoate, but *Alcaligenes latus*, *recombinant E. coli*, and *Azotobacter vinelandii* bacteria do not require nutrient limitation and acquire polyhydroxyalkanoate during the growth phase. It is an intercellular product and needs to be extracted from the bacterial cells followed by purification and application (Guo *et al.,* 2022; Anjum *et al*., 2016). One of the greatest advantages that polyhydroxyalkanoates possess over other biodegradable polymers is their ability to degrade under both aerobic and anaerobic conditions (Joel and Pieter, 2022). Similarly, polyhydroxybutyrate is the first reported short carbon length homopolymer of polyhydroxyalkanoates by Lemoigne in 1926 (Raza *et al.,* 2018). Polyhydroxybutyrate and other polyhydroxyalkanoates are produced and deposited intracellular as granules, accounting up to 90% of the dry weight of cells (Chloe *et al.,* 2022; Gouda *et al.,* 2013).

The physical properties of polyhydroxybutyrate are much similar to the conventional polypropylene and polyethylene; hence, it can be used in packaging applications. Polyhydroxybutyrate biocompatibility and non-toxicity makes it suitable to be used as a biomedical implant material and drug carrier in medical, surgical, and pharmacological disciplines. Biodegradable bottles, packaging materials, plastic and electronic accessories, furniture and coated paper have all employed polyhydroxybutyrate in their composition (Turco *et al.,* 2021; Garcia *et al.,* 2022; Raza *et al.,* 2018; Chen, 2009). The studies of Dubey and Mishra, 2021 reported that the bioplastic production might rise from 2 Metric tons per year in 2017 to 2.4 Metric tons per year in 2022 (Dubey and Mishra, 2021). Generally at commercial scale, polyhydroxyalkanoates are synthesized by pure substrates such as glucose, fructose, sucrose, etc., which encounter 40% of the production cost. The expense of the sugar substrate used for polyhydroxyalkanoates manufacture is one of the constraints limiting the commercial success of polyhydroxybutyrate and other polyhydroxyalkanoates production schemes. Researchers have focused on using low cost raw materials like glycerol, organic waste, wastewater, dairy waste, waste oil, and molasses for polyhydroxyalkanoates production (Kanzariya *et al*., 2023; Pagliano *et al*., 2020; Koller and Obruča, 2022). The use of inexpensive feedstock can make polyhydroxyalkanoate commercially viable. Annually, million tons of organic waste are generated by a diverse range of industries, where sugar industries contribute the most in terms of effluent (Li *et al.,* 2020; Bhuwal *et al.,* 2013). Cane molasses is a by-product of sugar industry, approximately 3 to 7 tons of cane molasses can be produced from 100 tons of fresh sugar cane which can be used as an inexpensive feedstock for polyhydroxyalkanoates production and may help in decreasing the polyhydroxyalkanoates production cost as well as disposal of waste (Khok *et al.,* 2019).

The objective of present work was to optimize PHA synthesis conditions using molasses as substrate by Alcaligenessp.NCIM5085 and suggest the suitable kinetic model for growth of cell biomass and PHA synthesis. The growth kinetics and PHA production kinetics differ from one to another bacterium and fermentation condition. The current study is unique because it is the first comprehensive examination of parameter optimization for cane molasses using *Alcaligenes* sp. *NCIM 5085* and its growth and polyhydroxyalkanoates synthesis kinetics.

Materials and Methods

Microorganism and culture media: *Alcaligenes* sp. NCIM 5085 was used as a polyhydroxyalkanoates precursor and procured from National Chemical Laboratory, Pune, India. The microorganism was maintained on nutrient agar media which consisted beef extract stored at 4°C (Himedia, Mumbai, India).

Preparation of cane molasses: The cane molasses was procured from Ganesh Sugar Industries, Vataria Gujarat, India. The sample was viscous dark brown, thick liquor, further analysis showed that it consisted high amount of sugar in the form of 2.3% glucose, 0.46% fructose and 28.19% sucrose. The pH of the sample was 4.5. Experiments were done with both pretreated and untreated cane molasses. Pretreatment was done by digesting cane molasses with 1.5 N H_2SO_4 at 90°C with stirring for 1 hr.

PHA synthesis and screening: To measure cell dry weight, polyhydroxyalkanoate, pH, and total reducing sugar at different time interval, a number of samples were prepared. Each cultured sample was picked at a desired time interval, centrifuged at 3000 rpm for 10 min at 4°C to separate cells from the solution. To account the cell biomass and polyhydroxyalkanoate visually, *Alcaligenes* sp. *NCIM 5085* was stained with Sudan black blue for screening where Sudan black blue was prepared with 70% ethanol and this solution was used to stain the heat-fixed smear, which was kept for 5 min as per procedure of (Mohanrasu *et al*.,2020).

PHA extraction and quantification: Polyhydroxyalkanoate concentration was determined by slightly modified method of Higuchi *et al*. (2016), where modified hypochlorite method was used for the extraction of polyhydroxyalkanoate. Polyhydroxyalkanoate quantification was done by the method of Silambarasan *et al.* (2021). The concentration of crotonic acid was measured in the experiment samples against calibration graph. Separated biomass was also analysed using Thermo Scientific Talos F200i transmission electron microscope. The supernatant was collected after centrifugation and analysed for total reducing sugars using Agilent HPLC model and refractive index (Shodex) detector, operated at 35°C. The samples were filtered through Bioflow 0.22 µm PES membrane cartridge and Sep-Pak classic Alumina A cartridges before analysis.

Kinetic modeling of cell growth: PHA synthesis begins intracellular with the formation of acetyl-CoA, which is produced through glycolysis. Condensation of two acetyl-CoA moieties yields acetoacetyl-CoA which is later reduced to 3-hydroxyalkanoates-CoA (3-HA-CoA). The polyhydroxyalkanoate polymerase enzyme catalyzes the polymerization of 3-HA-CoA into a polymer of 3-HA while releasing free CoA (Philip *at al.*, 2007). The bacterial cell growth curve contains four phases: the first, where bacteria adapt to their surroundings instantly after inoculation; the second log phase when cells develop and multiply at their fastest rates, and biomass and culture density both raise exponentially over time. During this stage of balanced growth, the rate of growth for every component of a cell is same. The rate at which variations in cell biomass concentration (X) are connected with the rate of biomass production according to the Malthus model is the rate at which the specific growth rate (µ) of cell biomass in batch processes is calculated. Malthus model provides a constant value µ (h^{-1}) for the relationship between the rates of biomass change through time. It is to be noticed that this model is useful only during the log development phase (Baei *et al.,* 2011).

The cell growth model can be classified in terms of substrate dependent model which is the Monod model, and substrate independent model, which is the logistic model. Monod model describes the relationship between specific growth rate and substrate consumption rate in the balanced growth period (log phase) in a batch process. In the third stationary phase growth rate of cell biomass is zero due to depletion of essential nutrient and substrate unavailability. In this phase, the cell requireds a small amount of energy for cell maintenance. Most polyhydroxyalkanoate is produced during the log phase and stationary phase of cell growth, while some bacteria are able to produce the maximum polyhydroxyalkanoate during the log phase, whereas others are able to accumulate polyhydroxyalkanoate during the stationary phase, and are able to make polyhydroxyalkanoate throughout both phases (Bhatia *et al.*, 2018). The fourth death phase: the cell number and culture density decreased rapidly (Liu *et al.*, 2014). A graph plotted between ln (X/X_m-X) vs t should give a straight line, where μ_m is equal to the slope of a line and X_0 was determined from the intercept. At stationary growth phase, the growth rate is zero *(dX/dt=0),* which is followed by a dead growth phase occur due to insufficient nutrients when the cell biomass weight is decreased.

Kinetic modeling of PHA production: The kinetics of PHA production involves PHA production during log and stationary phase. The PHA production can be related to 3 cases; one in which the PHA production occurs simultaneously with cell growth is called as growth associate product and the product formation rate is proportional to the cell growth rate; second in which PHA production occurs when cell growth rate is zero (stationary phase) is called non- growth associate product and the product formation rate is equal to the non-growth associated constant. In third case, the polyhydroxyalkanoate production occurs during slow cell growth rate and stationary phase is called as mixed growth associated product and the product formation rate is found by Luedeking–Piret kinetics model. In the present study, polyhydroxyalkanoate production is associated with mixed growth associated product. The rate of product generation is linearly dependent on both the instantaneous biomass concentration *X* and the growth rate *dX/dt* according to Luedeking–Piret kinetics (Shabnam *et al*., 2021).

Statistical analyses: All the experiments were performed in triplicate and the data were expressed as mean of three experiments with standard deviation. To select the best fitting kinetic model for all reactions, error analysis was carried out. The agreement between the estimated values and the experimental data was established by the coefficient of linear regression (R^2). The root mean square error (RMSE) was assessed by error analysis (Chauhan *et al*., 2015).

Results and Discussion

The influence of cane molasses concentration on PHA synthesis was studied to determine the inhibitory factors as well as optimum substrate concentration. At low substrate concentrations, the growth of an organism and polyhydroxyalkanoate content was less in both static and shaking conditions because the requisite amount of carbon source was not available that could not induce the stress required for polyhydroxyalkanoate synthesis. As the substrate concentration increased from 10 to 40 g l^{-1}, the production of biomass as cell dry weight increased from 0.4g l^{-1} to 2.45 g l^{-1} in static condition and an increase of 5-6% was observed with shaking fermentation for 48 hr of incubation as shown in Fig. 1a, b. The biomass, polyhydroxyalkanoate production and percent substrate consumption further decreased as the substrate concentration increased above 40 g l^{-1}, possibly a higher initial substrate concentration inhibited the cell growth. Hence, 40 g l^{-1} initial substrate concentration was considered optimum substrate concentration for polyhydroxyalkanoate synthesis. Furthermore, in order to observe the effect of static and shaking conditions, two sets of experiments were conducted where one media was kept in static conditions while the other was incubated at 180 rpm shaking condition at 35°C. As a result, 19.67 % higher cell dry

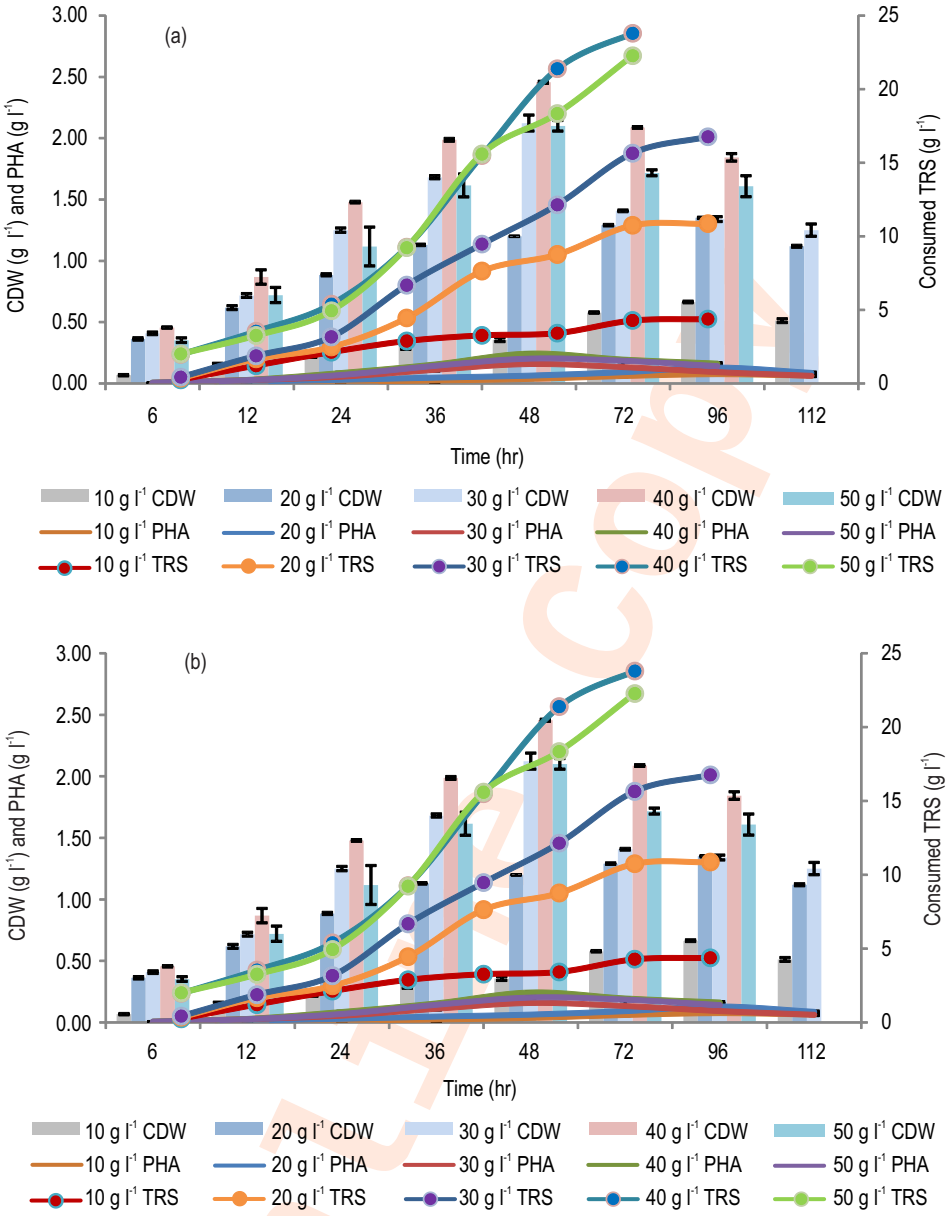


Fig.1: Effect of initial substrate concentration on the cell dry weight (CDW), polyhydroxyalkanoates yield (PHA) and consumed total reducing sugar (TRS) for untreated cane molasses under (a) static conditions and (b) shaking conditions.

weight, 40% higher polyhydroxyalkanoate content were obtained with shaking conditions (Fig. 2 a,b). It can also be pointed out that the shaking condition not only facilitated mass and oxygen transmission between phases but also ensured that the medium was homogeneous which enhanced the bacterial growth and PHA accumulation. Mohd. zahari *et al.* (2012) reported that shaking enhanced aeration rate in media and also increased the oxygen transfer rate which ultimately led to more PHA production. Whereas, the study of Zhou *et al.* (2018) reported that under shaking conditions the dissolved oxygen concentration was increased which led to more PHA production. A similar result was also obtained by Dubey and Mishra (2021), where the maximum polyhydroxyalkanoate content 0.24 g at shaking and 0.18 g at static condition were obtained when *H. daringness* and *H. ventrose* were grown on 3% and 4% algal biodiesel waste residue at 35°C, respectively. Under static and shaking growth conditions, as the incubation time increased, the cell dry weight and polyhydroxyalkanoates content increased upto 48 hr, beyond which a decrease was observed. due to depletion of nutrients and polyhydroxyalkanoate used by the microorganisms for growth. The highest biomass content was achieved with *Cupriavidus necator* after 42 hr, where citric molasses was used as substrate (Felipe *et al.,* 2019). Chaijamrus *et al.* (2008) also obtained maximum biomass of 5.7g l^{-1} after 45 hr of incubation when

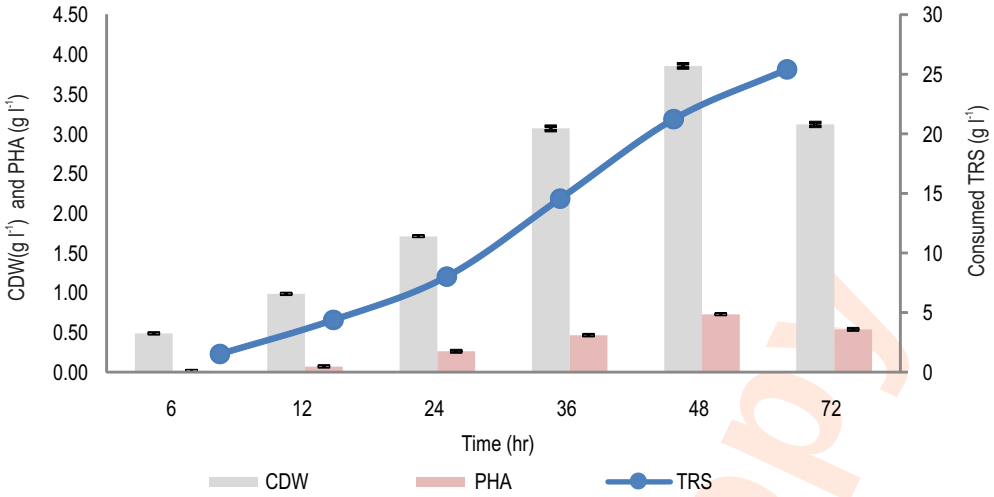


Fig. 2: Effect of pretreated cane molasses 40 g l^{-1} on cell dry weight (CDW), polyhydroxyalkanoates yield (PHA) and consumed total reducing sugar (TRS) under shaking conditions.

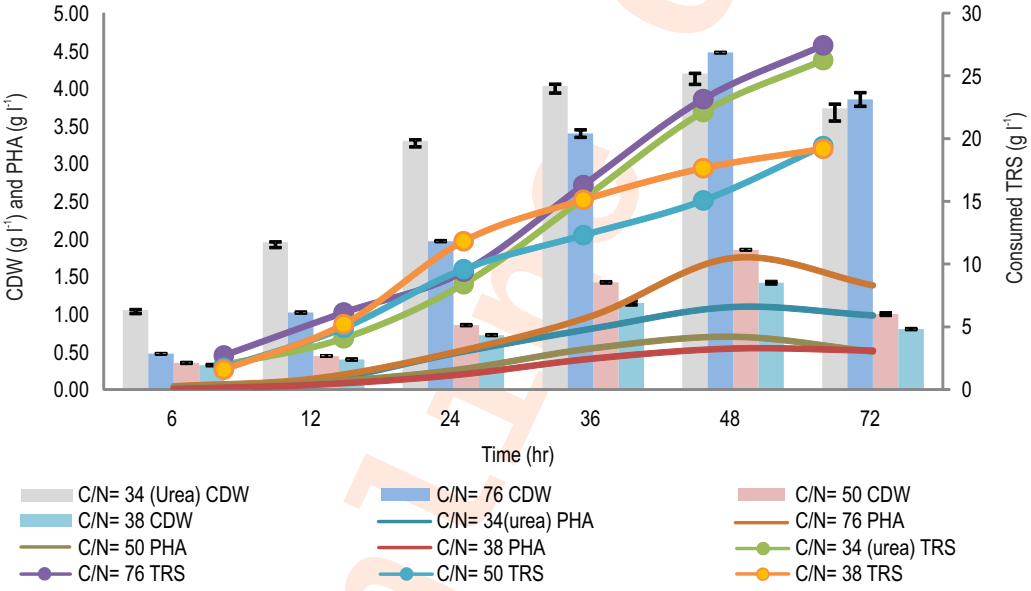


Fig. 3: Effect of C/N ratios on cell dry weight (CDW), polyhydroxyalkanoates yield (PHA) and consumed total reducing sugar (TRS) under shaking conditions with initial 40 g l^{-1} treated cane molasses.

Bacillus megaterium ATCC 6748 was grown on 4% cane molasses where 4% corn liquid syrup was used as a carbon and nitrogen source. In a separate batches of experiments, pretreated cane molasses at a concentration of 40 g l^{-1} cane molasses was used where higher cell dry weight was obtained and more polyhydroxyalkanoate was accumulated as compared to untreated cane molasses (Fig. 2). As *Alcaligenes* bacteria is unable to assimilate sucrose, it is essential to convert it into a monomer like glucose and fructose for bacterial utilization. After pretreatment, the glucose and fructose content increased to 16.5 % and 17.4 %from negligible quantity in untreated molasses. The acid treatment on cane molasses showed a positive effect on the polyhydroxyalkanoate production (Bhatia *et al.,* 2018; Khok *et al.,* 2019). Polyhydroxyalkanoate gets accumulated in the biomass under nutrient deficient conditions so it is important to observe the effect of nutrient concentration during fermentation.

A higher nitrogen uptake promotes biomass growth without stimulating polyhydroxyalkanoate and vice-versa (Arumugam *et al.,* 2019). Fig. 3 indicates the effect of ammonium

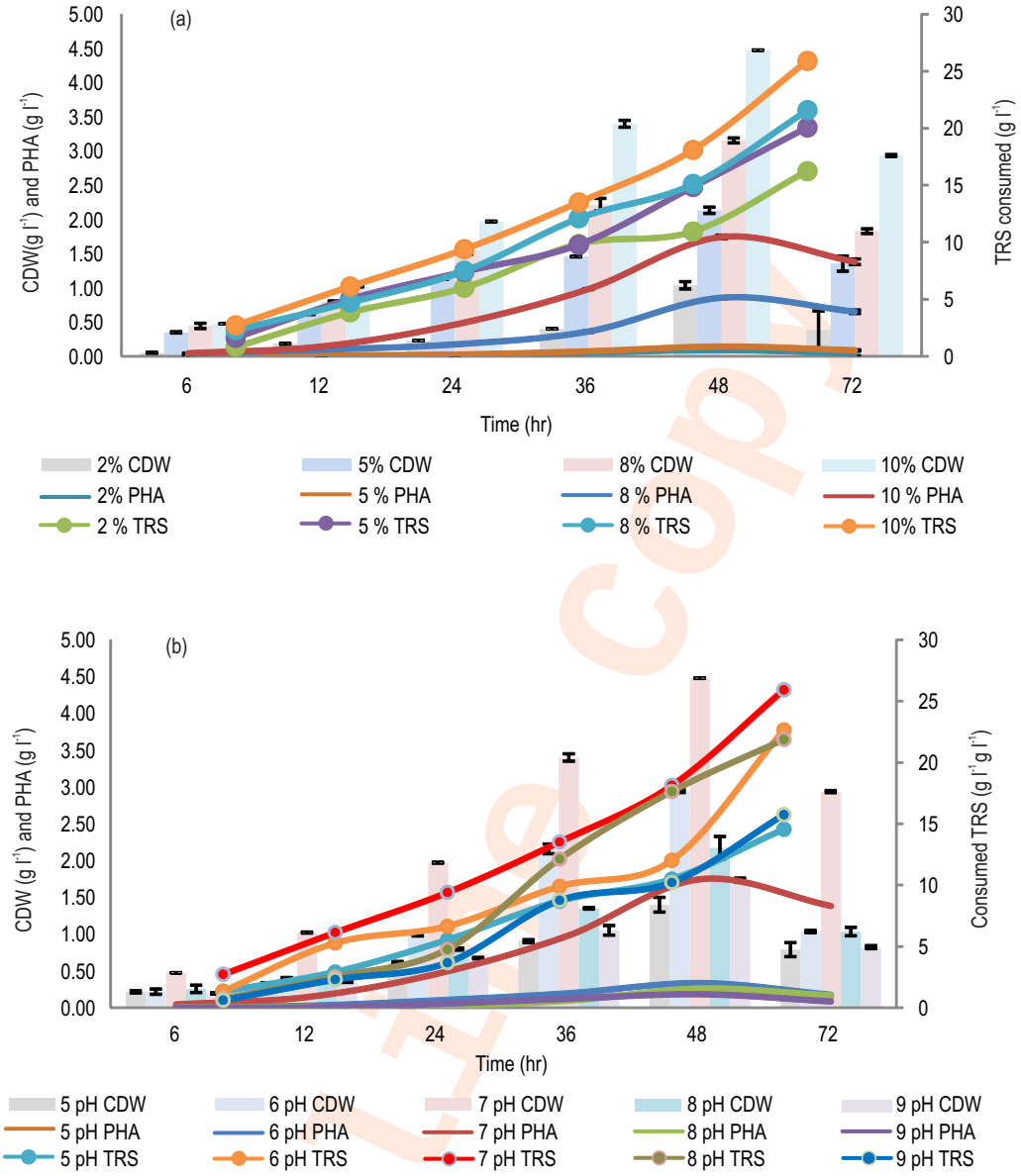


Fig. 4(a-b): Effect of (a) inoculum concentration and (b) initial pH on cell dry weight (CDW), polyhydroxyalkanoates (PHA) yield and consumed total reducing sugar (TRS) under shaking conditions with initial concentration of (40 g l^{-1}) treated cane molasses.

sulphate and urea as nitrogen sources on biomass and polyhydroxyalkanoate production. The maximum cell dry weight (4.48 g l^{-1}) and polyhydroxyalkanoate (1.75 g l^{-1}) were achieved when 68.56% substrate was consumed after 48 hr of incubation, when $(NH_4)_2SO_4$ was used as a nitrogen source. A less polyhydroxyalkanoate was observed when urea was used as a nitrogen source because bacteria hydrolyses urea into NH_3 and CO_2 and an increase of carbon in media leads to decrease polyhydroxyalkanoate accumulation whereas in case of $(NH_4)_2SO_4$, it is hydrolysed to $NH_4^+$and SO_4 –, therefore no additional carbon was generated, which actually supported polyhydroxyalkanoate accumulation. Another reason for higher production of polyhydroxyalkanoate colud be attributed to the presence of nitrogen in both the sources; urea contains 46% nitrogen and ammonium sulphate contains 21% nitrogen by molecular weight and it is known that PHA polymerizes within cell under nitrogen deficient conditions. We can conclude that enough nitrogen promotes cell development while inhibiting PHB accumulation because high PHB production was retrieved under nitrogen starvation. Therefore, in the experiments it was observed that initially upto 36 hr of incubation time, the biomass production was higher when urea was used as a nitrogen source, however,

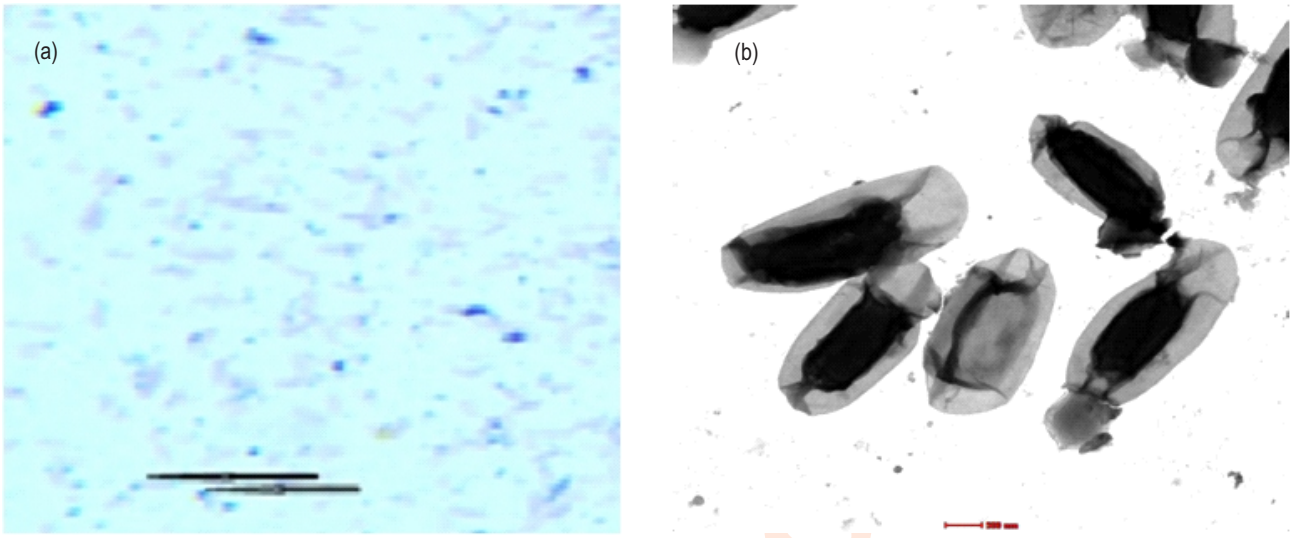


Fig. 5: (a) Microscopic observation of PHA granules produced in the bacterial strained with Sudan black blue (magnification 100X); (b) TEM analysis of *Alcaligenes* sp. NCIM 5085 viewed after 48 hr of inoculation in cane molasses production medium (magnification, 0.5 µm).

after 48 hr, the polyhydroxyalkanoate accumulation was higher when ammonium sulphate was used as a nitrogen source. Hence, $(NH_4)_2SO_4$ was found to be a better source of nitrogen having lesser ammoun of nitrogen. Further, it was found that the polyhydroxyalkanoate concentration was reduced on reducing the C/N ratio from 76 to 38 because the higher carbon content does not promote PHA yield but enhanced biomass accumulation (Fig. 3). Higher carbon content assists the cells ability to use tri-carboxylic acid cycle metabolic pathway to generate more energy and limiting the availability of acetyl-coA, which is the key substrate for polyhydroxyalkanoate synthesis. The initial concentration of microbes are the key ingredient in the fermentation of cane molasses and it will affect the biomass and further PHA yield. Fig. 4 (a) shows the effect of inoculum concentration on biomass and polyhydroxyalkanoate content. On increaseing the inoculum concentration from 2 to 10%, the biomass and polyhydroxyalkanoate content increased and the highest biomass (4.48 g l^{-1}) and polyhydroxyalkanoate content (1.75±0.03 g l^{-1}) was obtained at 10% inoculum concentration after 48 hrs of incubation. On further increasing the inoculum concentration, the biomass and polyhydroxyalkanoate content decreased. Therefore, 10% inoculum concentration was taken as optimum inoculum concentration. The inoculum volume helps in achieving optimal productivity in a shorter fermentation period while reducing the lag time, therefore it can be concluded from the result that a minimum concentration of bacterium is required to achieve the desired biomass production in a specified time. If the time is not a constraint then this parameter is in significant. In this study, it was observed that bacteria was extremely sensitive to any change in the pH of media. If it was deviated from neutral pH, cell growth as well as the polyhydroxyalkanoate accumulation were influenced. Fig. 4 (b) shows similar behaviour. It can be deduced from the findings that pH 7.0 was optimal for the growth and biosynthesis of PHA with 1.75±0.03 g l^{-1} at 4.48g l^{-1} cell dry weight was obtained where total reducing sugar consumed was 68.56±0.30%. At neutral pH, biosynthesis activities of bacteria was maximum which supports polyhydroxyalkanoate biosynthesis (Khamkong *et al.,* 2022; Gomaa *et al.,* 2004).

Transmission electron microscopy analysis (Fig. 5a) after 48 hr of inoculation in the production media revealed that the *Alcaligenes* sp. *5085* is a rod shape bacteria. Larger polyhydroxybutyrate granules were present near the bacterial cell membrane. Despite the fact that polyhydroxyalkanoate is produced in nitrogen-limited environments under stress and serves as a reserve material, microbes do not use the entire polyhydroxyalkanoate for growth and maintenance. Investigation was supported by Tian *et al.*(2005) where larger PHB granules were deposited inside the bacterial cell which observed with TEM analysis. Transmission electron microscopy study clearly demonstrated polyhydroxyalkanoate granules inside the cell. Fermentation of cane molasses with microbes takes longer time where different phases of microbes take place which ultimately influence the polyhydroxyalkanoate accumulation. The maximum specific growth rate was increased as the concentration of CM was increased. The concentration and time data was fitted with logistic and Monod growth kinetics model where constant µmax was 0.14 h^{-1}which was the highest value with R^2=0.99. The summary of all kinetic parameters for polyhydroxyalkanoate synthesis at various conditions is shown in Table.1. A comparison of experimental cell dry weight with the theoretical Logistic model is shown in Fig. 6 (a). The experimental results were found well fitted with the model at log phase growth kinetics; however, at the end of the stationary phase the experimental cell dry weight began to decline due to unavailability of nutrients whereas in the logistic model. Baei *et al.* (2011) reported the logistic equation

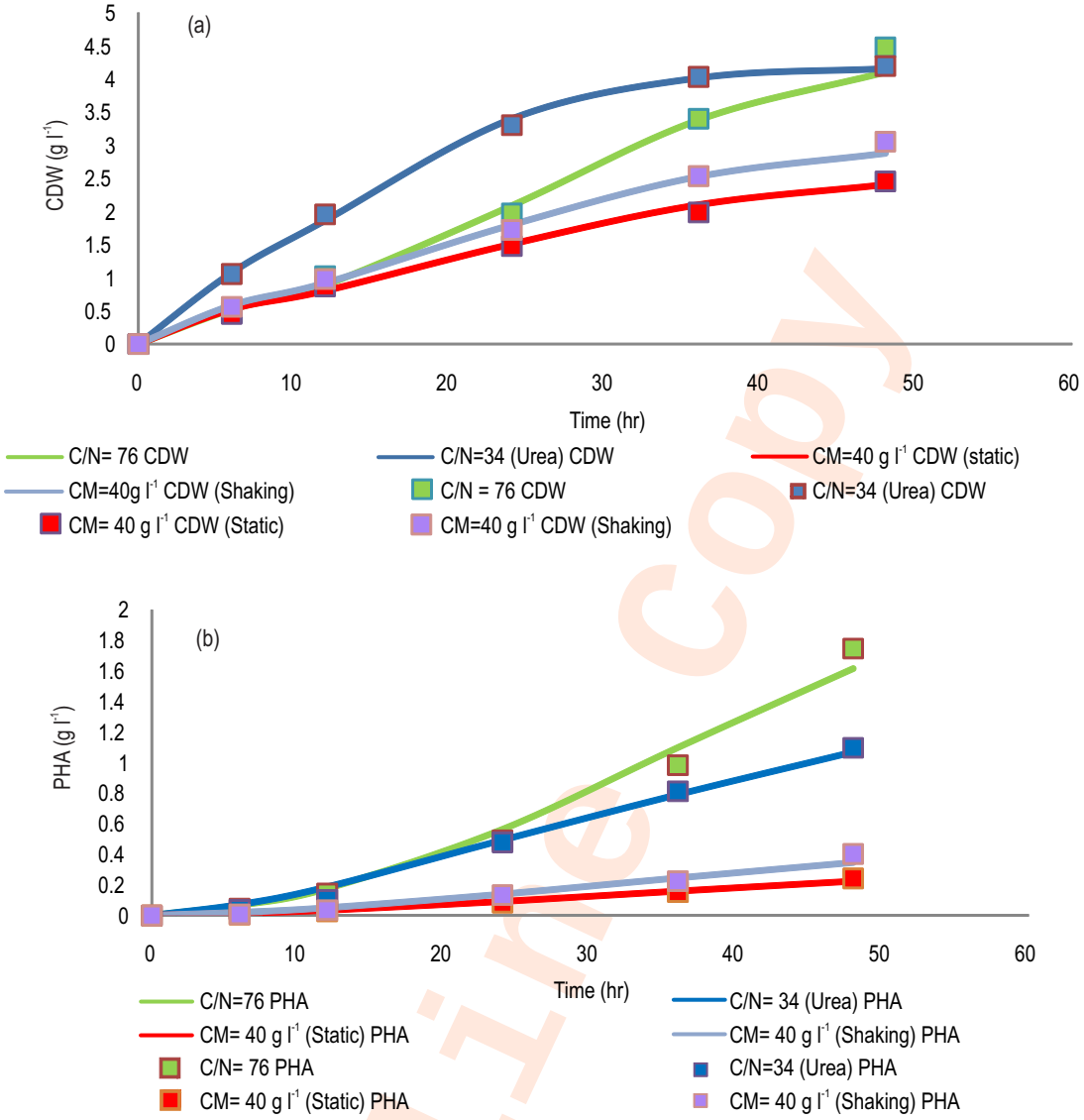


Fig. 6: Comparison of experimental (a) cell dry weight and (b) polyhydroxyalkanoates kinetics data and Model data for polyhydroxyalkanoates accumulation by *Alcaligenes*. sp. 5085 in shaking conditions with initial concentration of (40 g l^{-1}) treated cane molasses, C/N= 76 AS, initial pH 7 and 10 % inoculum.

(R^2=0.96) to predict biomass growth which fit well among all kinetic models for PHB production with *C. necator DSMZ 545* on selected substrate. Similar results were obtained by Dhanasekar *et al.* (2003), where logistic model was used on a mutant *A. vinelandii* consuming glucose in a batch reactor for P (3HB) production. Furthermore, increase in cane molasses concentration from 10 to 40g l^{-1}, increased in both models, however, it decreased after a certain concentration under static and shaking conditions. Gahlawat *et al.* (2013) reported that µm initially increased as the sucrose concentration increased up to 25 gl^{-1} after that increasing the sucrose concentration µm decreased where Polyhydroxyalkonte synthesis was done by *Azohydromonasaustralicaina* batch process. The μ_m also increased in the presence of nitrogen because it supports growth of biomass. The optimum inoculum concentration (10 v/v %) and optimum initial pH (7) further increased μ_m. However, data predicted by Logistic model fitted best out of two growth kinetic models where higher value of the coefficient of regression (0.999) and lowest RMSE value in the range of 0.008–0.16 was observed.

It was noticed that the experimental data fitted well with the model at log phase, however, at the end of stationary phase experimental PHA content decreased due to degradation of PHA.The growth associated constant α was obtained was much higher than β, which is a non-growth associated constant as shown in Table 1. It indicated that PHA production primarily

Table 1: Summery of kinetic parameters for PHA synthesis and growth model by *Alcaligenes* sp. NCIM 5085 batch fermentation

Experimental conditions		Logistic model				Monod model						Luedeking Piret model			
		μ_{max} (h^{-1})	X_0 (g)	R^2	RMSE (%)	μ_{max} (h^{-1})	Ks ($g\ l^{-1}$)	R^2	RMSE (%)	α	β	R2	RSME (%)
Without shaking and untreated CM	CM concentration 10 g l^{-1}	0.05	0.06	0.96	5.03	0.041	14.81	0.89	6.05	0.089	0.0013	0.97	0.33
	CM concentration 20 g l^{-1}	0.06	0.38	0.95	5.93	0.046	36.51	0.94	8.47	0.04	0.0010	0.89	0.24
	CM concentration 30 g l^{-1}	0.09	0.28	0.99	16.51	0.061	50.83	0.96	7.56	0.041	0.0016	0.96	0.65
	CM concentration 40 g l^{-1}	0.09	0.32	0.98	7.2	0.095	78.02	0.90	7.45	0.045	0.0021	0.93	1.01
	CM concentration 50 g l^{-1}	0.08	0.27	0.97	9.18	0.048	82.55	0.70	9.86	0.041	0.0026	0.96	3.92
Shaking and untreated CM	CM concentration 10 g l^{-1}	0.05	0.04	0.94	2.83	0.058	14.01	0.85	3.34	0.12	0.0018	0.93	0.56
	CM concentration 20 g l^{-1}	0.06	0.36	0.96	12.18	0.0706	39.37	0.92	13.48	0.02	0.0012	0.98	1.26
	CM concentration 30 g l^{-1}	0.09	0.27	0.97	16.97	0.071	52.10	0.87	11.97	0.06	0.026	0.84	2.40
	CM concentration 40 g l^{-1}	0.09	0.34	0.99	9.17	0.1	80.51	0.97	10	0.056	0.027	0.85	2.80
	CM concentration 50 g l^{-1}	0.08	0.34	0.98	6.52	0.057	83.263	0.87	2.84	0.07	0.022	0.92	1.94
Shaking and treated CM (40 g l^{-1} initial concentration)	N=0	0.10	0.28	0.98	15.04	0.25	70.24	0.89	15.58	0.1	0.004	0.98	1.72
	N=1 g l^{-1} Urea	0.14	0.53	0.99	6.83	0.16	92.73	0.97	8.59	0.086	0.0054	0.90	4.34
	N=1 g l^{-1} ammonium sulphate 7pH, 10% inoculum	0.10	0.29	0.99	9.21	0.17	83.95	0.93	10.40	0.21	0.008	0.92	4.03
	N=1.5 g l^{-1} AS	0.08	0.20	0.98	5	0.12	88.18	0.95	1.3	0.0079	0.0078	0.93	18.9
	N=2 g l^{-1} AS	0.08	0.08	0.97	4.36	0.089	55.95	0.98	6.94	0.0071	0.0079	0.93	16.74
	5 pH	0.07	0.15	0.99	0.87	0.037	64.74	0.97	5.78	0.2	0.004	0.98	12.13
	6 pH	0.09	0.1	0.99	10.4	0.92	64.74	0.93	11.19	0.081	0.0024	0.98	0.86
	8 pH	0.08	0.16	0.99	2.18	0.13	37.28	0.80	4.18	0.0156	0.002	0.96	1.65
	9 pH	0.07	0.11	0.95	8.94	0.081	73.20	0.81	9.84	0.07	0.0021	0.96	0.78
	8 % inoculum	0.08	0.30	0.98	11.21	0.0772	70.76	0.87	6.45	0.004	0.005	0.89	4.1
	5 % inoculum	0.07	0.27	0.97	7.68	0.038	0.75	0.75	8.75	0.064	0.0014	0.98	0.44
	2 % inoculum	0.07	0.04	0.91	16.18	0.035	0.77	0.77	13.65	0.14	0.0016	0.98	0.40

*N represents the nitrogen source or amount; CM: Cane molasses

followed the growth-related kinetic pattern with a slow rate of PHA production during the non-growth period. It is important to emphasize that kinetic parameters may alter due to variation in fermentation conditions like shaking, pH, nutrient concentration and substrate concentration (Sakthiselvan and Madhumathi, 2018; Batcha *et al.,* 2014). Similarly, the kinetic model for each experimental condition was generated separately as shown in Table 1. The data predicted by Logistic model was incorporated with Luedeking Piret model which satisfied the highest linear correlation 0.98 and lowest RMSE value in the range of 0.003-0.18.

Linear correlation coefficients (R^2) and root mean square error (% RMSE) were calculated to select the most suitable model for cell growth and PHA synthesis. A higher magnitude of linear correlation coefficient and lower magnitude of RMSE is desirable for better fitting of data. It was observed that an individual value of *RMSE* was smaller than 20% at all experimental conditions, for both kinetic models. Nevertheless, error values were higher with Monod growth kinetics model. It can also be observed from Table1 that linear correlation coefficient was more than 0.95 for the Logistic growth kinetic model, except for the lowest inoculum concentration, the value of linear correlation coefficient was less for Monod growth kinetic model than logistic growth kinetics model, therefore logistic growth model was selected as the best fitted model for cell growth kinetics for PHA synthesis.

The potential utilization of agriculture residue cane molasses as a substrate and nutritional sources are in the synthesis of Polyhydroxyalkanoate by *Alcaligenes* sp. *NCIM 5085* was addressed in this work. The optimized parameters and developed kinetics model for synthesis can be utilized to design large scale production.

Acknowledgments

Authors duly acknowledge the Gujarat Institute of Desert Ecology, Bhuj, India for their sincere support to extend their facilities for conducting the experiments. The authors would also like sincerely thank the Department of Science and Technology, India for funding the project (DST/TDT/WM/2019/064) under the Scheme Waste Management Technology. The research was carried out under the aforementioned project.

Authors' contribution: A. Guatum and S. Parikh: Designed the experiment; **R. Kanzariya:** Conducted the experiment and wrote the first draft of the manuscript; **A. Guatum:** Analysis of results; **S. Gautam:** Revised the final draft of manuscript and overall supervision of the work.

Funding: The research was carried out under the project of Department of Science and Technology, India for funding the project (DST/TDT/WM/2019/064) under the scheme Waste Management Technology.

Research content: The research content of manuscript is original and has not been published elsewhere.

Ethical approval: Not applicable.

Conflict of interest: Authors have no conflict of interest.

Data availability: Not applicable.

Consent to publish: All authors agree to publish the paper in *Journal of Environmental Biology.*

References

Akinwumi, A.R., N. Obinna, A.O. Ayeni, E. Ahuekwe and M.N. Chukwu: An overview of the production and prospect of polyhydroxyalkanote (PHA)-based biofuels: Opportunities and limitations. *Sci. Afr.,* **16**. e01233 (2022).

Anjum, M. N., and S. Tabasum: Microbial production of polyhydroxyalkanoates (PHAs) and its copolymers: A review of recent advancements. *Int. J. Biol. Macromol.,* **89**, 161–174 (2016).

Arumugam, A., T.S. Anudakshaini, R. Shruthi, K. Jeyavishnu, S. Sundarra and J.S. Sharad: Low-cost production of PHA using cashew apple (*Anacardium occidentale* L.) juice as potential substrate: Optimization and characterization. *Biomass Convers. Biorefin.,* **10**, 1167–1178 (2019).

Baei, S.M., G.D. Najafpour, H. Younesi, F. Tabandeh, H. Issazadeh and M. Khodabandeh: Growth kinetic parameters and biosynthesis of polyhydroxybutyrate in *Cupriavidus necator* DSMZ 545 on selected substrates. *Chem. Ind. Chem. Eng. Q.,* **17**, 1-8 (2011).

Batcha, A.F., D.M. Prasad, M.R. Khan and H. Abdullah: Biosynthesis of poly (3-hydroxybutyrate) (PHB) by *Cupriavidus necator* H16 from jatropha oil as carbon source. *Bioprocess Biosyst Eng.,* **37**, 943–951 (2014).

Beaulieu, M., Y. Beaulieu, J. Melinard, S. Pandian and J. Goulet: Influence of ammonium salts and cane molasses on growth of *Alcaligenes eutrophus* and production of polyhydroxybutyrate. *Appl. Environ. Microbiol.,* **61**, 165–169 (1995).

Bhatia, S.K., J.J. Yoon, H.J. Kim, J.W. Hong, Y. Hong, H.S. Song, Y.M. Moon, J.M. Jeon, Y.G. Kim and Y.H. Yang: Engineering of artificial microbial consortia of *Ralstonia eutropha* and *Bacillus subtilis* for poly(3-hydroxybutyrate-co-3-hydroxyvalerate) copolymer production from sugarcane sugar without precursor feeding. *Bioresour. Technol.,* **257**, 92–101(2018).

Bharathi, B., D. Gowdhaman and P. Venkatachalam: Isolation and Identification of polyhydroxybutyrate (PHB) producing *Bacillus cereus* BB613-A novel isolate. *Int. J. Chemtech. Res.,* **9**, 224-228 (2016).

Bhuwal, A.K., G. Singh, N.K. Aggarwal, V. Goyal and A. Yadav: Isolation and screening of polyhydroxyalkanoates producing bacteria from pulp, paper, and cardboard industry wastes. *Int. J. Biomat.,* **2013**, 752821 (2013).

Chauhan, G., K. K. Pant and K. D. P. Nigam: Conceptual mechanism and kinetic studies of chelating agent assisted metal extraction process from spent catalyst. *J. Indus. Engin. Chem.,* **27**, 373–383 (2015).

Chloe, B., G. Nathalie and A. Chahinez: Extending biopolyesters circularity by using natural stabilizers: A review on the potential of polyphenols to enhance Poly (hydroxyalkanoates) thermal stability while preserving its biodegradability. *Polym. Testi.,* **110**, 107561 (2022).

Chen, G.Q.: A microbial polyhydroxyalkanoates (PHA) based bio- and materials industry. *Chem. Soc. Rev.,* **38**, 2434-2446 (2009).

Chaijamrus, S. and N. Udpuay: Production and characterization of polyhydroxybutyrate from molasses and corn steep liquor produced by *Bacillus megaterium ATCC 6748*. *Agric. Eng. Int.*

CIGR J., **10**, 1-12 (2008).

Dai, Y., Z.Yuan, K. Jack and J. Keller: Production of targeted poly (3-hydroxyalkanoates) copolymers by glycogen accumulating organisms using acetate as sole carbon source. *J. Biotechnol.,* **129**, 489–497 (2007).

Dhanasekar, R., T. Viruthagiri and P. L. Sabarathinam: Poly (3-hydroxy butyrate) synthesis from a mutant strain Azotobactervinelandii utilizing glucose in a batch reactor. *Biochem. Eng. J.,* **16,**1-8 (2003).

Dubey, S. and S. Mishra: Efficient production of polyhydroxyalkanoate through halophilic bacteria utilizing algal biodiesel waste residue. *Front. Bioeng. Biotechnol.,* **9**, 624859 (2021).

Felipe A., F. Pavan, A. Pavan, L. Tassia, T. L. Junqueira, D.B. Junqueira, M.D.B. M. Watanabe, D.B. Watanabe, A. Bonomi, K. Luci, L. Quines, K. Quines, W. WillibaldoSchmidell, Schmidell- Glaucia M.F. G. Aragao and M.F. de Aragao: Economic analysis of polyhydroxybutyrate production by *Cupriavidus necator* using different routes for product recovery. *Biochem. Eng. J.,* **146**, 97-104 (2019).

Garcia, D., L. Carrillo, R. Balart, S.T. Giner and M.P. Arrieta: Innovative solutions and challenges to increase the use of Poly (3-hydroxybutyrate) in food packaging and disposables. *Eur. Polym. J.,* **178**, 111505 (2022).

Gahlawat, G. and A.K. Srivastava: Development of a mathematical model for the growth associated polyhydroxybutyrate fermentation by *Azohydromonas australica* and its use for the design of fed-batch cultivation strategies. *Bioresour. Technol.,* **137**, 98–105 (2013).

Gouda, M. K., E.A. Swellam and H.O. Sanaa: Production of PHB by a *Bacillus megaterium* strain using sugarcane molasses and corn steep liquor as sole carbon and nitrogen sources. *Microbiol. Res.,* **156**, 201-207 (2013).

Gomaa, E.: Production of polyhydroxyalkanoates (PHAs) by *Bacillus subtilis* and *Escherichia coli* grown on cane molasses fortified with ethanol. *Braz. Arch. Biol. Technol.,* **57**,145-154 (2014).

Guo, W., K. Yang, X. Qin, R. Luo, H. Wang and R. Huang: Polyhydroxyalkanoates in tissue repair and regeneration. *J. Eng. Regen.,* **3**, 24-40 (2022).

Higuchi, T. M., K. Morisaki, K. Toyooka and K. Numata: Synthesis of high-molecular-weight polyhydroxyalkanoates by marine photosynthetic purple bacteria. *PloS ONE*, **11**, e0160981 (2016).

Joel, B.M. and C.A. Pieter: Polyhydroxyalkanoate valorization beyond bioplastics: Opportunities as a circular carbon feedstock. *Curr. Opin. Green Sustain. Chem.,* **37**, 100656 (2022).

Kanzariya, R., A. Gautam, S. Parikh and S. Gautam: Formation of polyhydroxyalkanoates using agro and industrial waste as a substrate – a review. *Biotechnol. Genet. Eng. Rev.*, **14**, 1-40 (2023)

Kawaguchi, Y. and Y. Doi: Kinetics and mechanism of synthesis and degradation of poly (3-hydroxybutyrate) in *Alcaligenes eutrophus*. *Macromolecules*, **25**, 2324-2329 (1992).

Khamkong, T., W. Penkhrue and S. Lumyong: Optimization of production of polyhydroxyalkanoates (PHAs) from newly isolated *Ensifer* sp. strain HD34 by response surface methodology. *Processes*, **10**, 1632 (2022).

Khok Y.S., H.M. Hazwan and S. Baidurah: Biosynthesis of poly(3-hydroxybutyrate) (PHB) by *Cupriavidus necator* from various pretreated molasses as carbon source. *Biocatal. Agric. Biotechnol.,* **17**, 51-59 (2019).

Koller, M. and S. Obruča: Biotechnological production of polyhydroxyalkanoates from glycerol: A review. *Biocatal. Agric. Biotechnol.*, **42**, 102333 (2022).

Kulpreecha, S., A. Boonruangthavorn, B. Meksiriporn and N. Thongchul: Inexpensive fed-batch cultivation for high poly (3-hydroxybutyrate) production by a new isolate of *Bacillus megaterium*. *J. Biosci. Bioeng.,* **107**, 240-245 (2008).

Li, Q., Y. Zhou, C. Ke, Y. Bai, X. Liu and S. Li: Production of welan gum from cane molasses by *Sphingomonas* sp. FM01. *Carbohydr. Polym.,* **244**, 116485 (2020).

Liu Y., S. Huang, Y. Zhang and F. Xu: Isolation and characterization of a thermophilic *Bacillus shackletonii* K5 from a biotrickling filter for the production of Polyhydroxybutyrate. *J. Environ. Sci.,* **26**, 1453-1462 (2014)

MohdZahari, M. A., H. Ariffin, M.N. Mokhtar, J. Salihon, Y. Shirai and M.A. Hassan: Factors affecting poly (3-hydroxybutyrate) production from oil palm frond juice by *Cupriavidus necator* (CCUG52238(T)). *J. Biomed. Biotechnol.,* **2012**, 125865 (2012).

Mohanrasu, K., R. G. R. Rao, G. H. Dinesh, K. Zhang, G. S. Prakash, D. P. Song, S. Muniyasamyf, A. Pugazhendhih, J. Jeyakanthan and A. Arun: Optimization of media components and culture conditions for polyhydroxyalkanoates production by *Bacillus megaterium*. *Fuel*, **271**, 117522 (2020).

Pagliano, G., W. Gugliucci, E. Torrieri, A.Piccolo, S. Cangemi, F. A. Giuseppe, A. Robertiello, V. Faraco, O. Pepe and V. Ventorino: Polyhydroxyalkanoates (PHAs) from dairy wastewater effluent: Bacterial accumulation, structural characterization and physical properties. *Chem. Biol. Technol. Agric.*,**7**, 29 (2020). https://doi.org/10.1186/s40538-020-00197-1

Philip, S., T. Keshavarz and I. Roy: Polyhydroxyalkanoates: Biodegradable polymers with a range of applications. *J. Chem. Technol. Biotechnol.*, **82**, 233–247(2007).

Raza, Z.A., S. Abid and I.M. Banat: Polyhydroxyalkanoates: characteristics, production, recent developments and applications. *Int. Biodeterior. Biodegra.,* **126**, 45-56 (2018).

Shabnam S., M. Asadzadeh and D.B. Levin: Modelling cell growth and polyhydroxyalkanoate (PHA) polymer synthesis by *Pseudomonas putida* LS46 under oxygen-limiting conditions. *Curr. Biotechnol.,***11**, 39-50 (2021).

Silambarasan, S., P. Logeswari, R. Sivaramakrishnan, A. Pugazhendhi, B. Kamaraj, A. Ruiz, G. Ramadoss, and P. Cornejo: Polyhydroxybutyrate production from ultrasound-aided alkaline pretreated finger millet straw using *Bacillus megaterium* strain CAM12. *Bioresour. Technol.,* **325**, 124632 (2021).

Sakthiselvan, P. and R. Madhumathi: Kinetic evaluation on cell growth and biosynthesis of polyhydroxybutyrate (PHB) by *Bacillus safensis* EBT1 from sugarcane bagasse. *Eng. Agric.,* **11**, 145-152 (2018).

Tania Palmeiro-Sánchez, T.S. Palmeiro, O. Vincent, V. O'Flaherty and N.L. Piet, P.N. Lens and N.L. Lens: Polyhydroxyalkanoate bio-production and its rise as biomaterial of the future. *J. Biotechnol.,* **348**, 10-25 (2022).

Tian, J., A.J. Sinskey and J. Stubbe: Kinetic studies of polyhydroxybutyrate granule formation in *Wautersia eutropha* H16 by transmission electron microscopy. *J. Bacteriol. Res.,* **187**, 3814–3824 (2005).

Turco, R., G. Santagata, I. Corrado, C. Pezzella and S.M. Di: *In vivo* and post-synthesis strategies to enhance the properties of PHB-based materials: A review. *Front. Bioeng. Biotechnol.,* **8**, 619266 (2021).

Wisuthiphaet, N. and S.C. Napathorn: Optimization of the use of products from the cane sugar industry for poly (3-hydroxybutyrate) production by *Azohydromonas lata* DSM 1123 in fed-batch cultivation. *Process Biochem.,* **51**, 352-361(2016).

Zhou, Y., L.R. Han, H.W. He, B. Sang, D.L. Yu, J.T. Feng and X. Zhang: Effects of agitation, aeration and temperature on production of a novel glycoprotein GP-1 by *Streptomyces kanasenisi* ZX01 and scale-Up based on volumetric oxygen transfer coefficient. *Molecules*, **23**, 125 (2018).