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ORIGINAL ARTICLE

Low-cost production of PHA using cashew apple (*Anacardium occidentale* L.) juice as potential substrate: optimization and characterization

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Abstract
Polyhydroxyalkanoates are polyesters of R-hydroxyalkonic acids, prominently used as bioplastics on grounds of their complete biodegradable and environment-friendly characteristics. There is an upsurge in need of an alternative low-cost, renewable carbon source for the production of PHA for enhanced economic and to exert a positive impact on the industries. In the present work, cashew apple juice (CAJ) was supplemented as a carbon source for *Cupriavidus necator* to produce PHA. $(NH_4)_2SO_4$, NH_4Cl, NH_4NO_3 and $CO(NH_2)_2$, and $NaNO_3$ were tested and urea was found to be the best nitrogen source that supports optimal growth of the microorganism. The production process was then optimized using response surface methodology by incorporating the effects of total reducing sugar concentration, urea concentration, and inoculum size. Under optimized condition, the resulting PHA yield was found to be 15.78 g/L with total reducing sugar concentration of 50 g/L, inoculum size of 50 mL/L, and urea concentration of 3 g/L. FT-IR, NMR, TGA, and DSC analysis revealed the product to be a copolymer of hydroxybutyrate and hydroxyvalerate.

Keywords Polyhydroxyalkanoates (PHA) · Cashew apple juice (CAJ) · *Cupriavidus necator* · Response surface methodology (RSM)

1 Introduction

PHAs (polyhydroxyalkanoates) are the desired replacement for conventional plastics on the grounds that it has better biodegradability and physicochemical properties [1]. They are biocompatible, completely biodegradable, enantiomerically pure, exhibit piezoelectricity, highly stable with structural biodiversity, and good processability [2]. These properties are attributed due to the monomeric units of PHA (from 50,000 to 1,000,000 Da) [3]. SCL (small chain length) biopolymers exhibit a high degree of crystallinity (80%) with high melting point whereas MCL PHAs (medium-chain length polyhydroxyalkanoates) exhibit low crystallinity (25%) with a low melting point. SCL PHAs exhibit unique feature of being brittle and stiff compared to MCL PHAs which is highly flexible and elastic [4]. They are produced as granular inclusions in cell cytoplasm with 0.2 ± 0.5 mm in diameter [5] and are used for the production of bioplastics, medical implants, and drug delivery systems. PHAs are used across diverse disciples attributable to its potential application conferred due to its eco-friendly and physicochemical properties. They are used in medical implants for their biocompatible nature [6]. Evidence suggests that PHAs are used as drug carrier due to its better biodegradable characteristics [7]. Moreover, PHAs are considered to be the best stand-in for conventional petrochemical-based polymers for the production of bioplastics.

At the industrial scale, the cost of PHA production can be brought down by developing new strains capable of producing large quantities of PHAs. Another common methodology is to enhance existing fermentation and recovery process to gain value through the output low-cost [8]. Both are a mind-numbing process, as developing a strain requires genetic manipulation to maintain stability and upgrading a fermentation process requires additional cost in equipment design. One easiest method to decrease the production cost is to utilize cheap carbon feedstocks.

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Table 1 The comparison of PHA production from various carbon sources reported in the literature with the present work

Reference	Microorganism	Carbon source	Yield
Present work	*Cupriavidus necator*	*Anacardium occidentale*l	14.171 g/L
[23]	Phototrophic mixed cultures	Fermented cheese whey	0.60 Cmol PHA/Cmol*X* d
[24]	*Cupriavidus malaysiensis USMAA1020*	Synthetic medium	46 g/L 11.5 g/g Accumulation: 92%
[25]	*Haloferax mediterranei*	Macroalgal biomass subcritical hydrolysates	2.2 ± 0.12 g/L
[26]	*Pseudomonas pseudoalcaligenes*	Synthetic medium	5412.9 mM
[27]	Mixed culture	Nutrient-rich wet oxidation liquors	0.50 Cmol PHA/Cmol
[28]	Mixed culture	Heat pretreated waste sludge	0.23 mg COD/mg Xh
[29]	*Cupriavidus necator*	*Calophyllum inophyllum* oil	10.6 g/L
[30]	Activated sludge biomass with	Starch Industry waste water	PHA accumulating potential (PAP): 65% gPHA/gVSS
[31]	*Saccharophagus degradans*	Cellulose	Cell dry weight: 52.8%
[32]	*Bacillus thuringiensis*	Modified medium	Accumulation: 44.96%
[33]	*Pseudomonas putida KT2440*	Cider By-products	1.1 g/L

Several studies have reported the production of PHA using waste biomass and chemicals. PHA was produced from *Wickerhamomyces anomalous* VIT-NN01 utilizing sugar cane molasses and palm oil as a substrate with corn steep liquor [9]. Substrate concentration, nitrogen concentration, pH, time, temperature, and their effects were modeled and the optimized value was found to be 35 g/L, 2%, pH 8, 96 hands 37 °C respectively. A study compared PHA yield using acidified waste glycerol (AWG) and its derivatives in mixed culture and the result indicated high PHA production with AWG due to propanediol accumulation. The effect of carbon to nitrogen ratio on the simultaneous synthesis of extracellular polymeric substances (EPS) and PHA was investigated using *Hlaloferax* Mediterranean [10]. The EPS (exopolysaccharides) productivity and biomass growth increased with an increase in C/N ratio. It was found that PHA content per cell dry weight (CDW) elevated under nitrogen-limited conditions whereas volumetric productivity increased with increase in nitrogen concentration. *Halomonas halophila* was capable of accruing PHA up to 82% of its cell weight when grown in different inexpensive [11]. *Cupriavidus necator* belongs to Betaproteobacteria, a class of gram-negative microorganisms. It is considered to be the most versatile microorganism for PHA production, with the aptness to accumulate biopolymers up to 90% of its cell mass. In addition, they can metabolize various carbon sources such as lactic acid, starch, and fructose. Production of PHA has been generalized by the extensive use of divers of vegetable oils and simple carbon sources. Some of the substrates are glucose [12], fructose [13], mannitol [14], starch [15], sucrose [16], xylose [17], rubber seed oil [18], glycerol [19], date seed oil and date molasses [20], cheese whey [21], sugar cane bagasse [22], rice straw [2], etc. Table 1 summarizes reports on PHA production from various low-cost carbon sources and their corresponding yield. Though manifold feedstocks are reported for PHA production, its cost can be a major factor that can exert influence on the overall production of PHAs [34]. Only a few studies were reported for renewable and low-cost precursors. Demand for alternative low-cost substrates capable of reducing manufacturing cost has risen to reduce the price of the product. Cashew (*Anacardium occidentale*) is an evergreen tree found in most of the tropical countries. India is a major cashew producing countries in the world [35] with a total cultivation area of 1335.6 thousand Ha (hectare) (Horticultural statistics (2017), department of agriculture). Majority of cashew apples are left to rot after separating cashew nut. However, 12% of them are used for producing concentrated juices and desserts [36]. CAJ (Cashew apple juice) was used as a carbon substrate to produce oxalic acid (OA) using *Aspergillus niger* [37]. Parameters such as cashew apple juice concentration, pH, $NaNO_3$ concentration, time, and methanol concentration were optimized using response surface methodology and maximum OA yield was found to be 122.68 g/L under CAJ 150 g/L, pH of 5.4, $NaNO_3$ of 2 g/L, and methanol of 1% volume. Another study reported the

supplementation of CAJ with ammonium sulfate to produce mannitol using two *Leuconostoc* strains and achieved a product yield of 95% using *Leuconostoc mesenteroides* B-512F. These bacteria utilized inorganic nitrogen source (ammonium sulfate) along with the amino acids present in CAJ as an alternative to conventional complex nitrogen sources like yeast extract. From the lens of novelty, this the first comprehensive study for production of PHA using cashew apple juice as a renewable and low-cost carbon source for *Cupriavidus necator*. The best nitrogen source for the microbial fermentation was selected based on initial experiments. The process parameters such as nitrogen concentration, total reducing sugar concentration, and inoculum content were optimized using response surface methodology (RSM). Finally, the produced PHA was analyzed by FT-IR, NMR, TGA, and DSC analysis.

1.1 Metabolic pathway

Figure 1 shows the biosynthetic pathway for PHA production from *Cupriavidus necator* using cashew apple juice as a carbon substrate. The strain *Cupriavidus necator* utilizes glucose as a carbon source to synthesize PHA. Glucose follows the glycolysis pathway to generate acetyl-CoA as the precursor for PHA synthesis. The conversion of acetyl-CoA to acetoacetyl-CoA is catalyzed by 3-ketothiolase (also known as Pha A) combining two molecules of acetyl-CoA to yield acetoacetyl-CoA. This product is further catalyzed by acetoacetyl-CoA reductase (also known as Pha B) to produce 3-hydroxybutyryl-CoA which on further reaction with Pha C produces PHA. The yield of PHA is depended on the availability of acetyl-CoA which is further depended on the availability

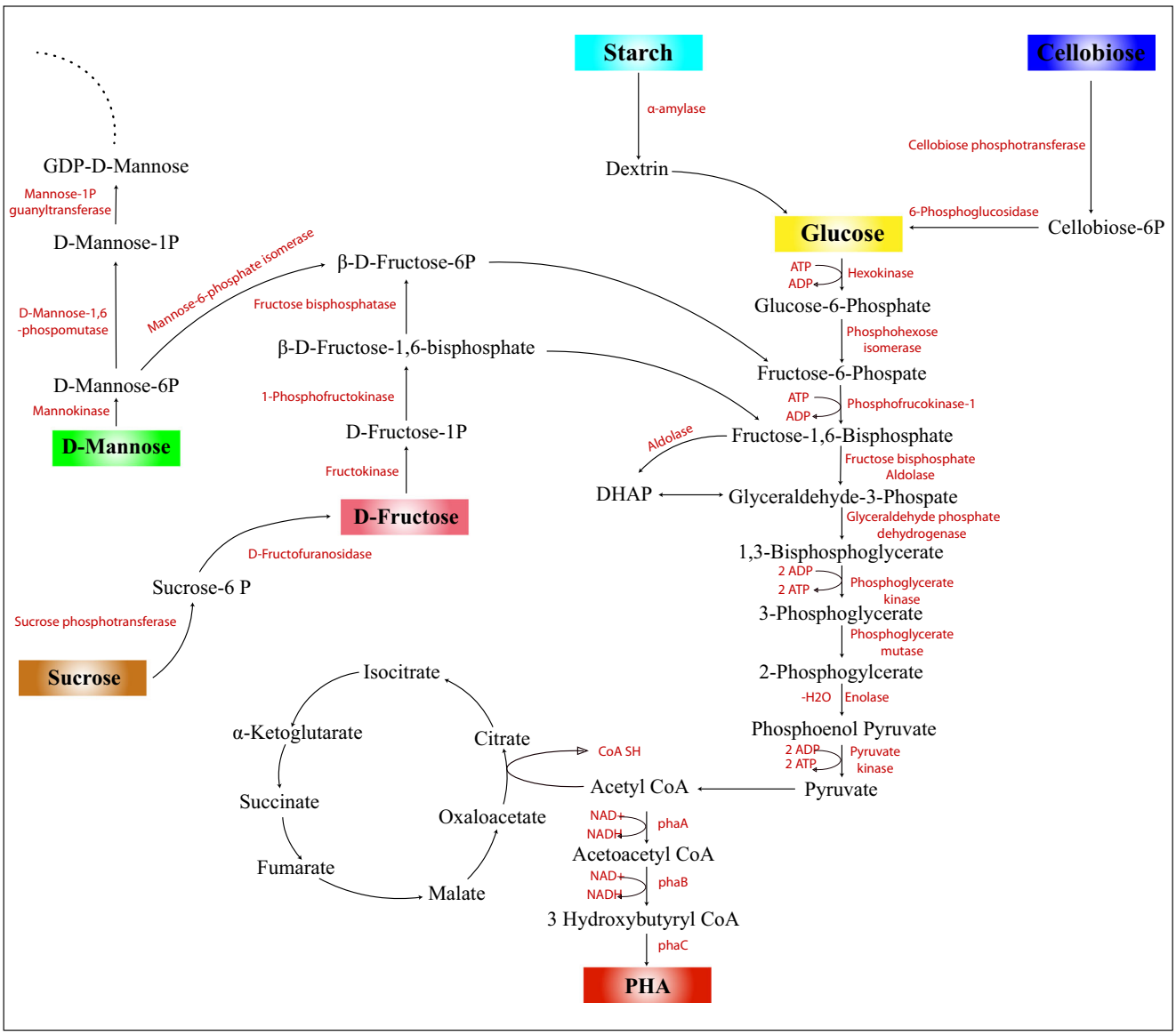

Fig. 1 The biosynthetic pathway for the production of PHA from *Cupriavidus necator* using cashew apple juice

of glucose. Conversion of fructose to fructose-1,6 bisphosphate is achieved by two main enzymes in *Cupriavidus necator* namely ketohexokinase (fructose to fructose-1-phosphate) and 1-phosphofructokinase (fructose-1 phosphate to fructose-1,6 bisphosphate). Fructose-1,6 bisphosphate further metabolizes to PHA by the following glycolysis. Conversion of mannose to mannose-6 phosphate (enzyme mannokinase) to fructose-6 phosphate (enzyme mannose -6 phosphate isomerase) is achieved through mannose metabolism, were fructose -6 phosphate follows glycolysis to yield PHA. Enzymes sucrose phosphotransferase and beta-fructofuranosidase converts sucrose to fructose and follows fructose metabolism to yield PHA. Cellobiose and starch are broken down to glucose, which is utilized for the synthesis of PHA via glycolysis pathway.

2 Materials and methods

2.1 Media and bacterial strain

Cupriavidus necator (MTCC 1954) was obtained from IMTECH, Chandigarh, India. The microorganism was maintained in a modified mineral medium containing 4.8 g/L Na_2HPO_4, 3.0 g/L $(NH_4)_2SO_4$, 0.8 g/L $MgSO_4.7H_2O$, 0.02 g/L $CaCl_2.2H_2O$, 2.0 g/L KH_2PO_4, 1.0 g/L, NaCl, 0.05 g/L NH_4Fe(III)citrate, and trace element solution SL6 (25 mL/L) [12]. Cashew apples were obtained from an agricultural farm near Pudukkottai district, Tamilnadu, India (10° 41′ 33.4″ N, 79° 04′ 40.2″ E), and were used as a carbon source. The cashew apple juice fermentation medium was comprised of diluted cashew apple juice, containing 27.35 g/L of fructose and 22.47 g/L of glucose.

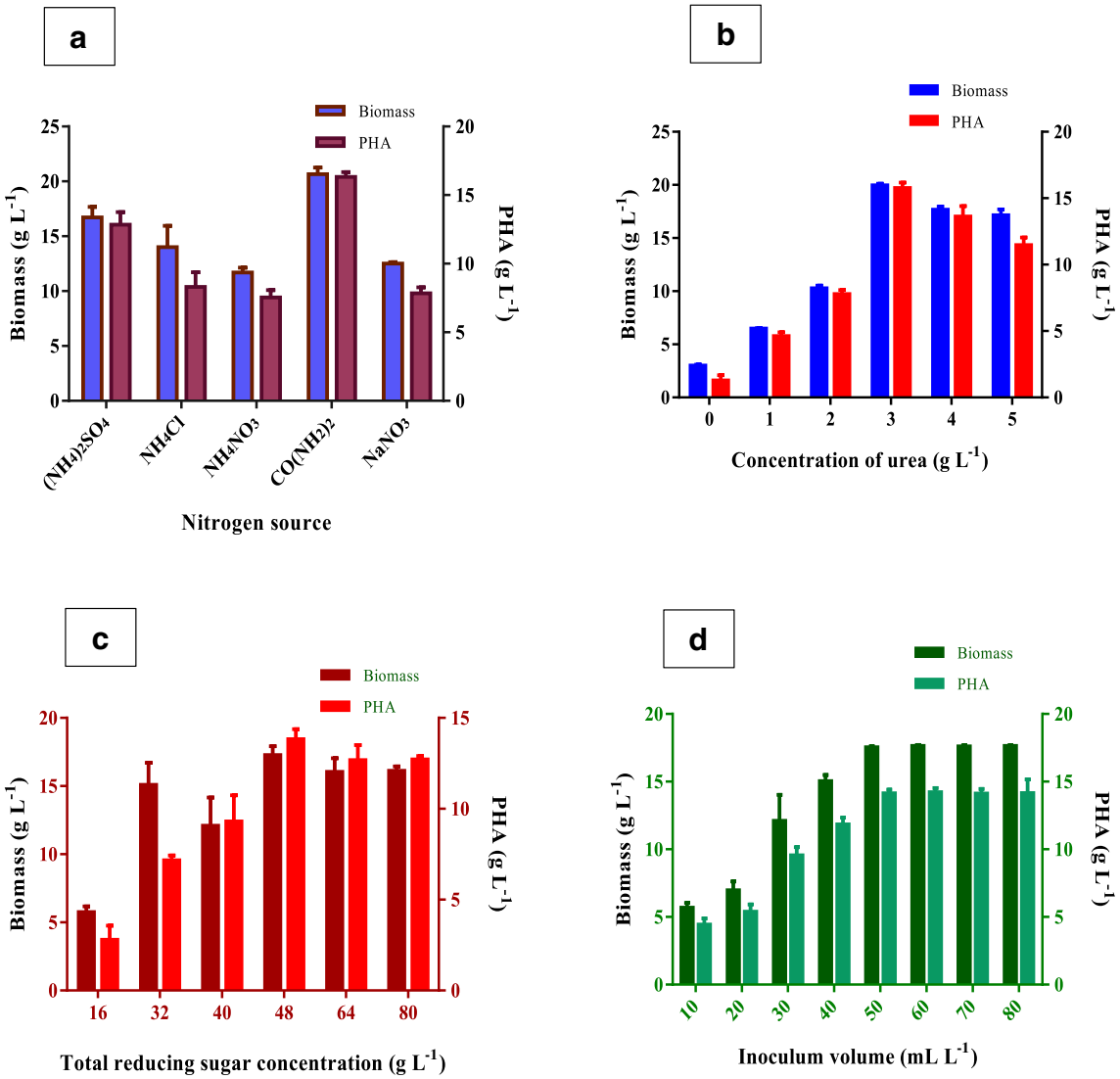

Fig. 2 **a** Effect of different nitrogen source on biomass (g/L) and PHA (g/L) accumulation in *Cupriavidus necator*. **b** Effect of urea concentration on biomass (g/ L) and PHA (g/L) accumulation for *Cupriavidus necator*. **c** Effect of total reducing sugar concentration on biomass (g/L) and PHA (g/L) accumulation for *Cupriavidus necator*. **d** Effect of inoculum size on biomass (g/L) and PHA (g/L) accumulation for *Cupriavidus necator*

Table 2 Experimental results based on central composite design for PHA production

RunOrder	PtType	Blocks	Total reducing sugar (g/L)	Urea content (g/L)	Inoculum size (mL/L)	Experimental PHA (g/L)	Predicted PHA (g/L)
1	1	1	20	0	20	0.48	0.57
2	1	1	80	0	20	3.70	3.58
3	1	1	20	6	20	4.75	3.51
4	1	1	80	6	20	8.60	6.51
5	1	1	20	0	80	4.60	4.34
6	1	1	80	0	80	3.40	3.96
7	1	1	20	6	80	7.50	7.28
8	1	1	80	6	80	8.99	6.89
9	− 1	1	20	3	50	8.77	9.73
10	− 1	1	80	3	50	12.12	11.04
11	− 1	1	50	0	50	8.90	8.63
12	− 1	1	50	6	50	11.40	11.56
13	− 1	1	50	3	20	10.65	10.83
14	− 1	1	50	3	80	13.20	12.90
15	0	1	50	3	50	15.32	13.88
16	0	1	50	3	50	14.86	13.88
17	0	1	50	3	50	15.78	13.88
18	0	1	50	3	50	13.20	13.88
19	0	1	50	3	50	14.30	13.88
20	0	1	50	3	50	15.10	13.88

2.2 Production and extraction of PHA

A total of 1.3 g of nutrient broth powder was added to the medium and was made to 100 ml and transferred to Erlenmeyer flask. A loop full of bacterial strain was inoculated in the nutrient-rich cashew apple juice and incubated at 30 °C for 2 days at 110 rpm. Different nitrogen sources such as $(NH_4)_2SO_4$, NH_4 Cl, NH_4NO_3 and $CO(NH_2)_2$, and $NaNO_3$ were added one at a time in a medium containing 50 g/L of total reducing sugar and 10 mL/L of inoculum. The optimum concentration of the selected nitrogen source was determined by varying the concentration from 1 to 5 g/L. Total reducing sugar concentration was varied from 16 to 80 g/L by changing the amount of CAJ in the media. Effect of inoculum volume on PHA production and CDW (cell dry weight) was studied by adding different volumes of inoculum (10–80 mL/L) to the media. Fermentation in shake flasks followed by centrifugation at 8000 rpm for 15 min resulted in the formation of cell pellets. The pellets were dried to determine the CDW. Acid extraction of PHA was identified to be the best method for extraction of PHA; hence, H_2SO_4 was used as the solvent for extraction [38]. The cells pellets were treated with 3.5% (v/v) H_2SO_4 at 80 °C for 6 h and centrifuged for 15 min at 8000 rpm. The pellet was resuspended in 0.5 N NaOH to set the final pH to 10, followed by 1 h of 3% sodium hypochlorite centrifugation. The pellet was resuspended in distilled water and centrifuged. The final form of the pellet was free-dried and stored.

2.3 Response surface methodology

In bacterial fermentation processes, RSM is extensively used for the optimization of process parameters to find near ideal condition for maximum yield [39]. A three-level three-factor central composite design (CCD) was employed, which includes 8 factorial runs, 6 axial runs, and 6 replicate runs at the center. $N = 2^n + 2n + N_c = 2^3 + 2 \times 3 + 6 = 20$. Twenty runs were drawn up and data were obtained. The response pattern and optimization combination of the variables was studied by central composite design with the three process variables. The response was used to develop an empirical model that correlated the response to the three process variables using a second-degree polynomial. Three parameters were chosen for RSM study [40]. They are namely total reducing sugar concentration, inoculum volume, and urea concentration their range was 20–80 g/L, 0–6 g/L, and 20–80 mL/L respectively.

2.4 PHA biosynthesis in a batch reactor

Batch fermentation study was carried out in a 3 L (2.5 L working volume) stirred tank Lark borosilicate glass bioreactor (height/diameter = 1.67) having integrated pH and dissolved oxygen controller (New Brunswick BioFlo 110). The inoculum was prepared in nutrient broth and incubated at 30 °C and 110 rpm for 18 h in a shake flask. 57.3 mL/L of culture was

Table 3 Analysis of variance for PHA production

Source	DF	Seq SS	Adj SS	Adj SS	*F*	*P*
Regression	9	389.293	389.293	43.255	45.44	0.000
Linear	3	61.183	61.183	20.394	21.42	0.000
Total reducing sugar (C_{TRS})	1	11.475	11.475	11.475	12.05	0.006
Urea concentration (C_{NIT})	1	40.651	40.651	40.651	42.7	0.000
Inoculum volume (C_{IN})	1	9.057	9.057	9.057	9.51	0.012
Square	3	320.927	320.927	106.976	112.38	0.000
C_{TRS}*C_{TRS}	1	243.581	33.593	33.593	35.29	0.000
C_{NIT}*C_{NIT}	1	66.152	39.503	39.503	41.5	0.000
C_{IN}*C_{IN}	1	11.194	11.194	11.194	11.76	0.006
Interaction	3	7.184	7.184	2.395	2.52	0.118
C_{TRS}*C_{NIT}	*1*	*1.376*	*1.376*	*1.376*	*1.45*	*0.257**
C_{TRS}* C_{IN}	1	5.749	5.749	5.749	6.04	0.034
C_{NIT}*C_{IN}	*1*	*0.058*	*0.058*	*0.058*	*0.06*	*0.810**
Residual error	10	9.519	9.519	0.952		
Lack-of-fit	5	5.394	5.394	1.079	1.31	0.388
Pure error	5	4.125	4.125	0.825		
Total	19	398.813				
R-Sq = 97.61%	R-Sq(pred) = 88.08%			R-Sq(adj) = 95.46%		

* Statistically insignificant (p>0.05

inoculated into the mineral medium comprising 3.58 g/L of urea and 51.94 g/L of total reducing sugars at pH 7.

2.5 Characterization of PHA

The freeze-dried pellet was subjected to FT-IR (Fourier transform infrared spectroscopy) studies in order to identify and characterize the structure of biopolymer. The pellet was dissolved in chloroform and added to KBr. The spectra were recorded in the range 4000 to 400 cm^{-1} after evaporation of the solvent. Nuclear magnetic resonance spectroscopy gives us an accurate structural elucidation of the biopolymer concerned. Both carbon-13 (C13) and proton NMR (H1) are the prerequisites of any structural identification steps [41]. The spectra were recorded at 75 and 300 MHz respectively using deuterated chloroform as the solvent. TGA (thermogravimetric analysis) and DSC (differential scanning calorimetry) were carried out using a Netzsch 204 thermal analysis system [42]. The rate of temperature rise in the apparatus was kept constant at 10/min for a range of 0 to 800 °C under an inert nitrogen atmosphere.

3 Results and discussion

3.1 Effect of nitrogen sources

Nitrogen source is necessary for producing PHA from the cells. Higher nitrogen uptake leads to an increase in biomass growth without inducing PHA production whereas lower nitrogen concentration is optimum for PHA accumulation. In this study, different nitrogen sources such as $(NH_4)_2SO_4$, NH_4Cl, NH_4NO_3, $CO(NH_2)_2$, and $NaNO_3$ were evaluated for PHA production. The effect of these nitrogen sources on biomass (g/L) and PHA (g/L) accumulation in *Cupriavidus necator* is shown in Fig. 2a. The nitrogen sources which supported growth and PHA production was found to be in the order: $CO(NH_2)_2 > (NH_4)_2SO_4 > NH_4Cl > NH_4NO_3 > NaNO_3$. For the maximum cell dry weight (20.6 g/L), PHA yield was found to be 16.33 g/L by utilizing $CO(NH_2)_2$ nitrogen source was provided in the growth medium. Hence, urea was found to be the best source of nitrogen for this study. Similar results were obtained by Ko-Sin Ng et al. [43], employs fructose as the carbon source and ammonium sulfate as the nitrogen source for PHA production. The optimal values of the selected variables were C = 20 g/L, N = 1.5 g/L, P = 8.75 g/L, and pH 7.5. A maximum PHB production of 4.6 g/L.

3.2 Effect of urea concentrations

In the fermentation process, the effect of urea concentration on PHA yield was studied by varying its concentration from 0 to 5 g/L. Nygaard et al. [44] reported maximum PHA yield at 1.5 g/L of ammonium sulfate as a nitrogen source for *Curpiavidus necator*, by varying the nitrogen concentration from 1 to 5 g/L. After centrifugation, pellet CDW was weighed and noted down. From the experiment, 3 g/L of urea yielded 19.96 g/L of CDW and 15.77 g/L of PHA. Figure 2b depicts the effect of urea concentration on biomass (g/L) and PHA (g/L) accumulation in *Cupriavidus necator*. A study

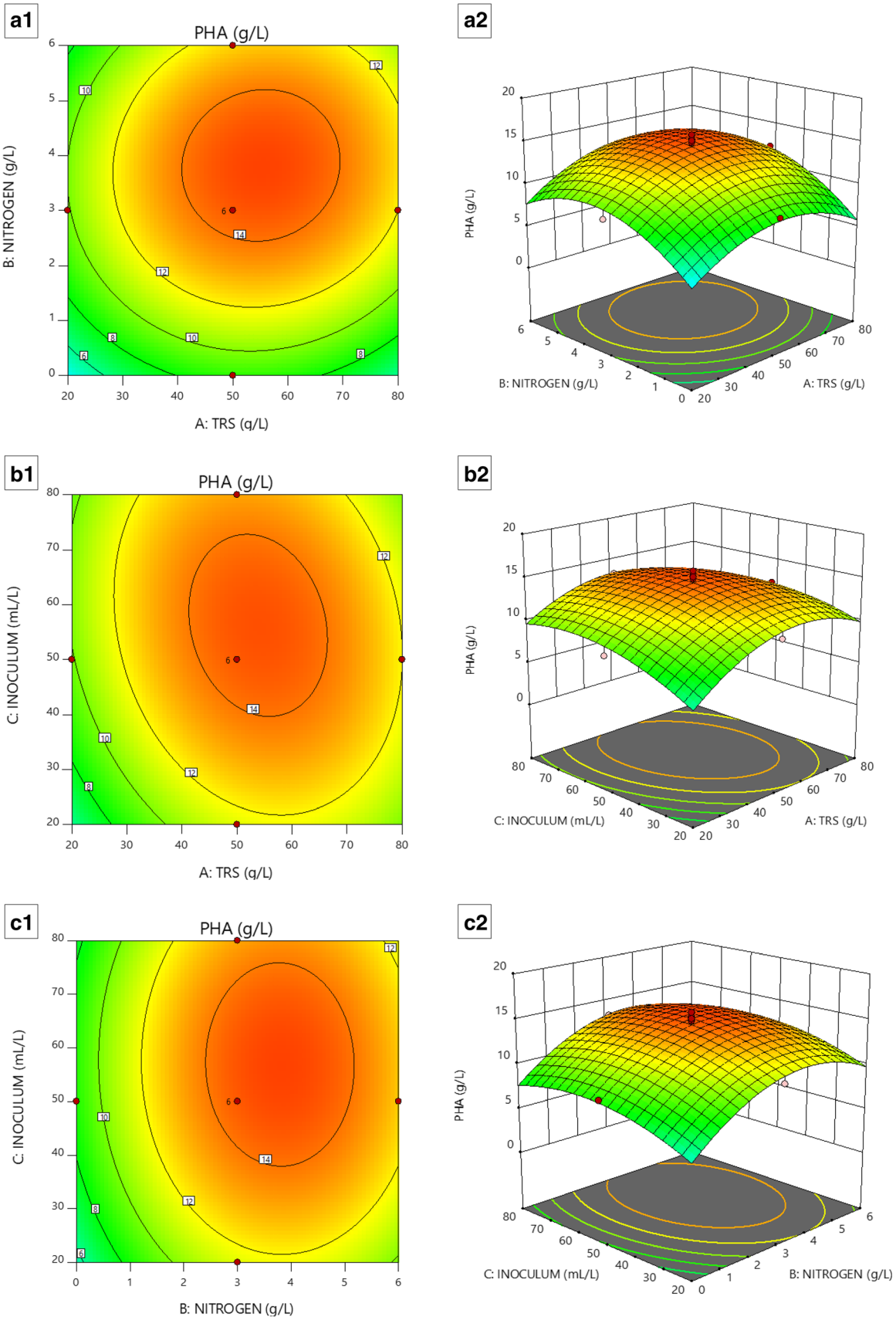

Fig. 3 Interaction effect of (A1&A2) nitrogen and TRS on PHA yield. (B1&B2) inoculum and TRS on PHA yield. (C1& C2) Inoculum and nitrogen on PHA yield. (TRS, total reducing sugars)

reported 80% of PHA yield with 0.54 g/L of urea in the medium containing palmolein and fructose [45].

3.3 Effect of TRS concentrations

The total reducing sugar concentrations (TRS) maintained at 16 to 80 g/L in a medium containing 10 mL of inoculum. Figure 2c shows that the PHA and CDW increased until reducing sugar concentration was 50 g/L after which it shows a decreasing trend. A total of 48 g/L of reducing sugars in the fermentation medium resulted in 17.27 g/L of CDW and 13.83 g/L of PHA, with the total PHA yield of 80%. Aramvash et al. [13] used 20 g/L fructose to produce PHA and Haas et al. [12] used 50 g/L of glucose to produce 3.10 g/L of PHA in a membrane bioreactor.

3.4 Effect of inoculum concentrations

Inoculum ranging from 10 to 80 mL/L was taken to determine the effect on PHA production. Figure 2d shows the effect of inoculum volume on biomass (g/L) and PHA (g/L) for *Cupriavidus necator*. Results show that inoculum volume had no effect on PHA synthesis but affected the CDW. The CDW increased with inoculum volume and remained constant after 50 mL/L. The maximum CDW was found to be 17.54 g/L with 14.14 g/L of PHA for 50 mL/L of inoculum. A.

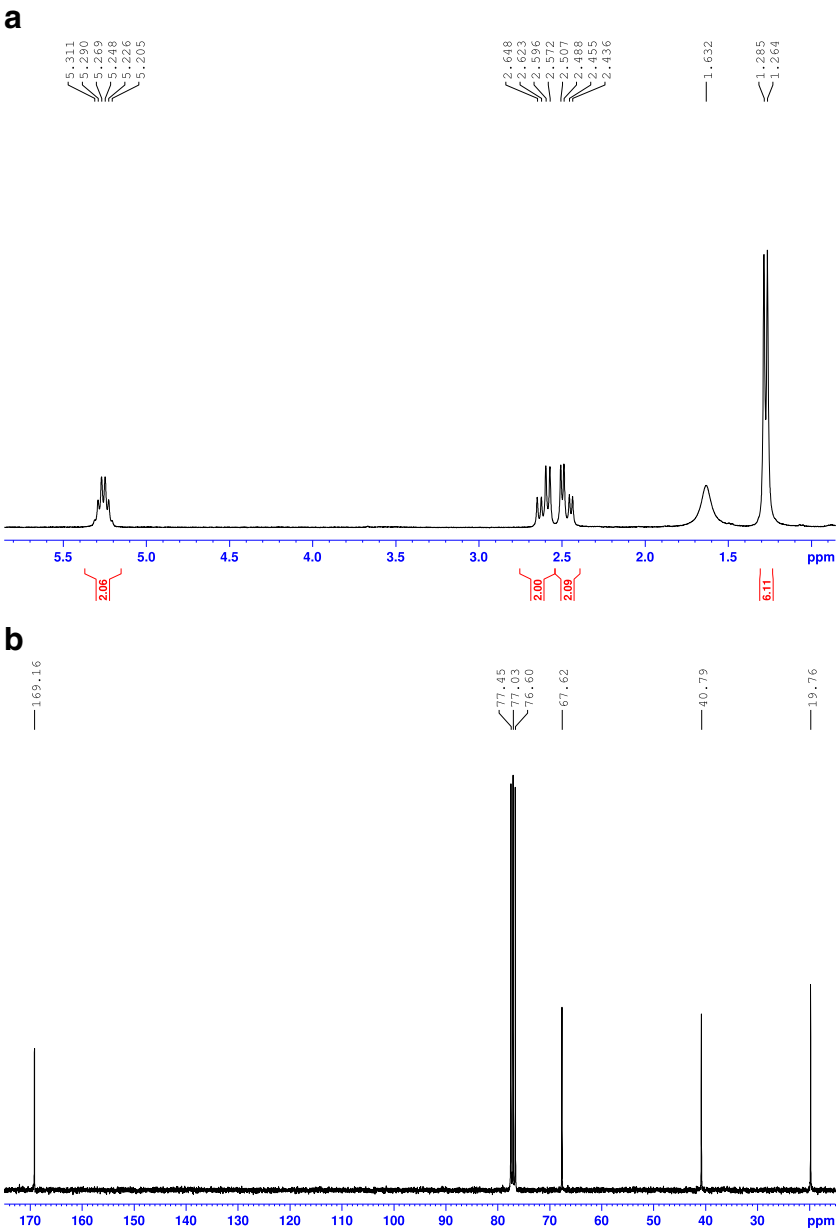

Fig. 4 **a** 1H NMR and **b** 13C NMR of extracted PHA produced by *Cupriavidus necator*

Arumugam et al. have studied the effect of inoculum concentrations on PHA yield and got similar results [36].

3.5 Process optimization

After performing 20 experiments suggested by the three-level three-factor CCD (central composite design) (Table 2), the following quadratic equation was generated (Eq. 1):

$$\text{PHA yield(g/L)} = \left\{ \begin{array}{c} -11.86 + (0.457 \times C_{TRS}) + (3.02 \times C_{NIT}) + (0.31 \times C_{IN}) \\ -(0.0039 \times C_{TRS}^2) - (0.42 \times C_{NIT}^2) - (0.0023 \times C_{IN}^2) \\ -(9.42 \times 10^{-4} \times C_{TRS} \times C_{IN}) \end{array} \right\} \quad (1)$$

Table 3 shows the results obtained from the analysis of variance (ANOVA). The mean and square effects were significant (p value less than 0.05), as evident from the regression analysis. In interaction effects, the total reducing sugar and inoculum concentration (TRS * INO) were found to be significant than others. The R^2 and adj. R^2 values were found to be 97.61% and 95.46% respectively which indicates the accuracy of the model [45]. Optimum conditions for maximum yield of PHA (15.78 g/L) were the concentration of total reducing sugar of 50 g/L, inoculum of about 50 mL/L with a urea content of 3 g/L. On solving the model, optimum conditions were found to be 51.7 g/L of total reducing sugar, 56.79 mL/L of inoculum concentration, and 3.59 g/L of urea concentration. The predicted optimum values were used to solve the equation and the PHA yield as was found to be 14.25 g/L. Experiments were conducted using the predicted optimum conditions and the results were found to be in confirmation with the model. The surface and contour plots confirm the same as shown in Fig. 3. Similarly, Box-Behnken design (BBD) was used in a study to optimize parameters for production of PHA from carbon dioxide sequestering *Bacillus cereus* SS105. The total biomass after optimization was found to increase from 1.05 to 1.10 g/L [46]. Similarly, date seed oil at a concentration of 10 g/L gave 7.6 g/L of PHA [20]. In another study, cheese whey was used as a production medium for *H. Mediterranei* biomass yielding 7.54 g/L poly-3 hydroxybutyrate-co-3hydroxyvalerate [21]. These reports show the importance of renewable feedstocks in polyhydroxyalkanoates production. Due to the increasing cost of carbon sources, there is a significant shift in PHA production from conventional glucose-based substrates to renewable feedstocks.

3.6 Characterization

The ^{1}H NMR spectrum of the polymer shows peaks at 1.264 and 1.285 ppm indicating the methyl group of hydroxyvalerate (HV) and hydroxybutyrate (HB) respectively (Fig. 4a). The peak at 1.632 ppm corresponds to a methylene group of HV. Signals at 2.436, 2.455, 2.488, 2.507, 2.572, 2.596, 2.623, and 2.648 ppm correspond to a methylene group of HV and HB. Signals at 5.205, 5.226, 5.248, 5.269, 5.290, and 5.311 ppm correspond to the methane group of HV and HB respectively. The peak at 7.265 represents $CDCl_3$. The analysis showed that the product is a copolymer of HB and

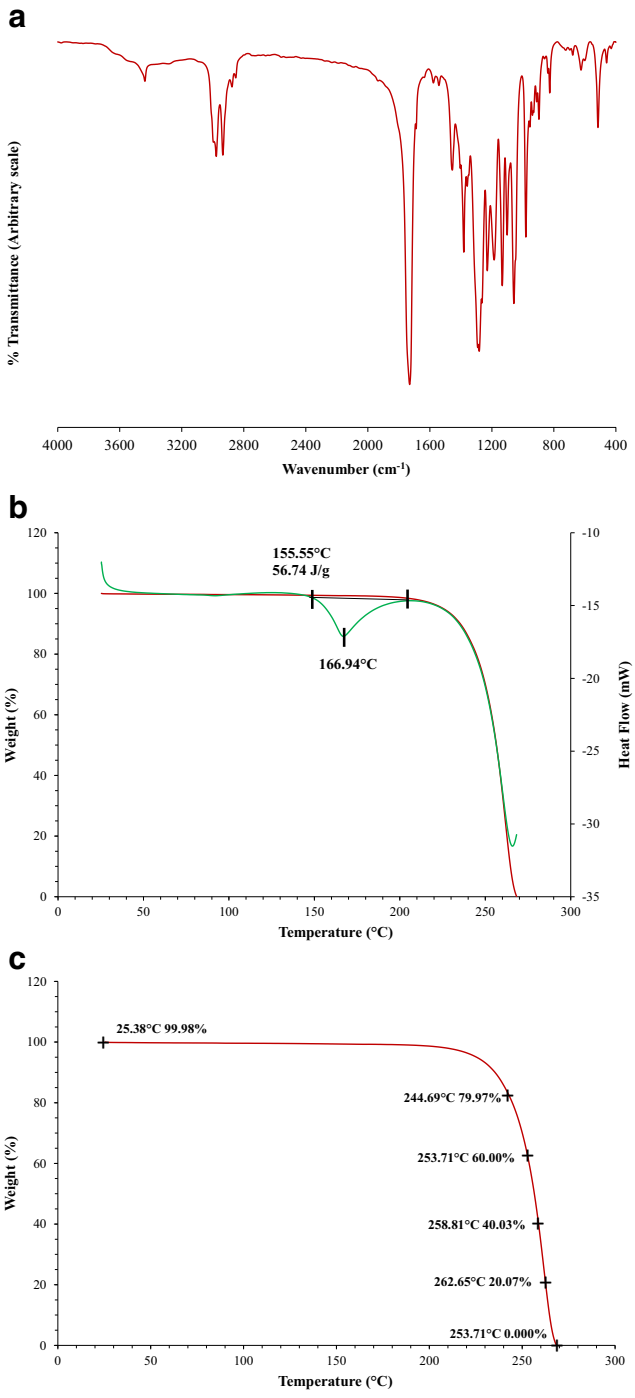

Fig. 5 FT-IR (**a**), TGA plot (**b**), and DSC curves (**c**) for polyhydroxyalkanoates produced by *Cupriavidus necator* using cashew apple juice as a carbon source

HV (poly (3-hydroxybutyrate–co-3-hydroxyvalerate)). Similar NMR spectra were reported in some studies [9, 40]. ^{13}C NMR of polymer produced by *Cupriavidus necator* showed signals at 67.62 ppm and 77.60–77.45 ppm corresponding to the methine group (-CH-) group of HB (hydroxybutyrate) and HV (hydroxyvalerate) respectively (Fig. 4b). The signal at 40.79 ppm and 19.76 ppm represented the methylene (-CH_2) group and methyl (-CH_3) group of hydroxybutyrate. The signal at 169.16 represented the ester (O-CH-) carbonyl (—C—) group. Similar results were also found in the literature [9, 40]. The Fourier-transform infrared spectra of the obtained polymer are shown in Fig. 5a. The three main functional groups present in PHA are CH, CH_2, and C=O. The bands at 2935.14 cm^{-1} and 2977.55 cm^{-1} represent CH vibration. The peak observed at 1730.06 cm^{-1} representing the C=O stretching was found to be the characteristic peak of PHA [29]. The peak at 2851.89 cm^{-1} corresponds to –CH_2-CH_3. Peaks between 1132 and 1281 cm^{-1} represented C-O-C stretching. The methyl and methylene peaks at 1455.04 cm^{-1} and 1381.04 cm^{-1} are observed in the spectra respectively. The peak at 1057.88 cm^{-1} refers to the C-O bond of the ester group. The obtained results were in agreement with previously reported studies [22, 47]. Figure 5b shows the thermal degradation profile of PHA in TGA when exposed to heat ranging from 0 to 300 °C under nitrogen atmosphere. The first significant weight loss was found to occur at 244.7 °C (79.97%) followed by complete degradation of the polymer at 268.4 °C. In an experiment, maximum thermal degradation points of the extracted PHB were found to be 287 °C and 266 °C respectively [23]. Another study reported the effects of poly-3 hydroxyvalerate incorporation in poly-3 hydroxybutyrate and found a slight decrease in degradation temperature (from 278.7 to 273.5 °C). In the present study, the degradation temperature was found to be close to the previously reported results. From the DSC curves (Fig. 5c), the melting point and glass transition temperature were found to be 155.5 °C and 166.9 °C respectively. Similarly, PHB obtained from *Bacillus aryabhattai* was found to be stable in the range of 30–140 °C with a melting point as 170 °C [48, 49].

4 Conclusion

Cupriavidus necator was used to produce PHA from high reducing sugar containing cashew apple juice as a low-cost substrate. At industrial levels, the price of carbon feedstock has a direct impact on biopolymer production. This present study proves to be a breakthrough in the field of biopolymer technology as cashew apple juice is a low-cost substrate compared to the reported feedstocks. Synthesis of high value-added bioproduct from cheap carbon feedstock like cashew apple juice reduces the price for producing PHA. Under the optimized condition, the PHA yield was recorded as high as 15.78 g/L. Results from FT-IR, NMR, TGA, and DSC studies confirmed the presence of a copolymer of hydroxybutyrate and hydroxyvalerate in the synthesized polymer. Therefore, the present study achieves the aim to utilize this cheap and non-edible feedstock for the production of a beneficial product.

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Compliance with ethical standards

Conflict of interest The authors declare that they have no conflict of interest.

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