Available Online at http://www.recentscientific.com

ISSN: 0976-3031

*International Journal of Recent Scientific Research*
*Vol. 6, Issue, 6, pp.4857-4860, June, 2015*

*International Journal of Recent Scientific Research*

RESEARCH ARTICLE

# BIOREMEDIATION OF COFFEE HUSK THROUGH POLYHYDROXYALKANOATES (PHA) PRODUCTION FOR A GREENER ENVIRONMENT

**S. Poorna Chandrika[1], D. Sabarinathan[1] and K. Preethi[1*]**

Department of Microbial Biotechnology, Bharathiar University, Coimbatore, Tamilnadu, India

**ARTICLE INFO**

***Article History:***

Received 2nd, May, 2015
Received in revised form 10th, May, 2015
Accepted 4th, June, 2015
Published online 28th, June, 2015

***Key words:***

Non-degradable plastics, Polyhydroxyalkanoates, coffee husk, 16SrRNA sequencing, Nile blue agar, Sodium hypochlorite method, FT-IR analysis.

**ABSTRACT**

Global risk of petroleum based non- degradable plastics and solid wastes produced from different industries have increased in an alarming rate urging for an effective alternative. The present work focuses on the production of Polyhydroxyalkanoates (PHA) utilizing coffee husk as a cheap organic substrate. PHA producing bacteria was isolated from coffee husk dumped soil and screened in Nile blue agar plates, they were subjected to 16SrRNA sequencing. The organism was further inoculated into two different media - Simple Media and Mineral Salt Media. Further coffee husk with media components was compared with coffee husk extract with different percentages of glucose (2% and 4%). The ability of the organism to utilize coffee husk alone without any media supplementation was also studied. PHA was extracted by Sodium hypochlorite method and quantified by UV spectrometry. FT-IR analysis was done to authenticate PHA production.



## INTRODUCTION

Solid waste management being the major problem concerning global environment is taken as an important plot for researches to work on. The occurrence of PHAs in bacteria has been known since 1920s, when Lemoigne reported the formation of poly 3-hydroxybutyrate (PHB) inside bacteria (Lemoigne, 1976).PHA are the polymer's used by microorganisms as carbon and energy sources under unfavorable growth conditions, such as Nitrogen, Phosphorous, Sulfur, Magnesium or Oxygen limitations or in the presence of excess carbon source (Shang *et al*., 2003). Then they utilize it by depolymerizing them to water soluble monomers with the help of depolymerase enzymes. Its beneficiary that the organism producing the polymer itself has the ability to degrade it and thus making the plastic more eco-friendly.

In order to produce PHA to meet industrial needs, cheaper substrates have to be explored. As PHA is one such polymer which could be completely relied on replacing conventional plastics. The present work focuses on using solid waste from coffee processing industry to be exploited as organic substrate for PHA production. Coffee industry plays a very important role throughout the world economy. Currently ranked sixth in the world in coffee production, India grows about 3 lakh tones annually (Narasimha *et al*).Tons and tons of coffee industry wastes are produced each year which includes coffee husk and coffee pulp. Coffee husk waste (CHW) discarded as bio-organic waste, from coffee industries, is rich in carbohydrates (Subbiah and Abidha, 2011) which are an additional advantage for the production of PHA. These wastes are either burnt to ash or dumped into soil or water bodies creating many environment risks. Hence utilizing these wastes could help in solving two problems i) a cheap substrate for PHA production ii) Bioremediation of Coffee husk.

In the present work, coffee husk hydrolysate obtained by the acid hydrolysis was investigated as media for bacteria to synthesize PHA. Simple media components like Glucose, Peptone in different concentrations supplemented to the substrate were also studied for better yield of PHA. The extracted PHA was characterized by UV spectrophotometer and FTIR analysis.

## MATERIALS AND METHODS

### *Substrate and sample collection*

Coffee husk (substrate) and coffee husk dumped soil (for isolation of PHA producing bacteria) was collected from AVT Coffee Company, Vellakinar Village, Coimbatore, Tamil Nadu. The substrate and soil samples were taken in sterile ziplock covers and aseptically brought to laboratory and maintained at

**Corresponding author:* **K. Preethi**
Department of Microbial Biotechnology, Bharathiar University, Coimbatore, Tamilnadu, India

4˚C, the husk was collected in sterile bags and transferred to the laboratory.

***Substrate preparation***

Acid hydrolysis of coffee husk was done using HCl(1% V/V) and autoclaved at 121°C for 30 min. pH of the acid post hydrolysate was adjusted to 6.8±0.2 pH and again filtered through filter paper. The glucose content was estimated by Anthrone method (Hedge *et al.,* 1962).

***Screening for PHA producers***

The pure cultured bacterial isolates on nutrient agar slants were streaked on Nile blue agar plates. Nile blue A was dissolved in DMSO and added to the agar medium. The plates were then incubated for 72-96 hours and examined under UV illuminator where the PHA accumulators show bright fluorescence (Spiekermann *et al.*, 1999).

***16SrRNA Sequencing for bacterial identification***

The positive PHA accumulators were given specific codes for screening and their biochemical analysis were done. 16SrRNA sequencing of the best PHA producing bacteria was done by extracting the DNA by chloroform phenol method (Bowtell and Sambrook, 2002) and amplified with 16SrRNA primer and the PCR product was subjected to 16SrRNA sequencing. Sequences from 16SrRNA were compared with other sequences in Gene Bank (http://www.ncbi.nlm.nih.gov).

***Fermentation Process***

The ability of the PHA producing bacteria to grow in different media with and without coffee husk extract was analysed. Simple Media(SM) with 1% glucose, peptone, sodium chloride and 0.5% yeast extract and Mineral Salt Media (MSM) NaCl - l % (w/v) , $Na_2HPO_4$ - 0.37 % (w/v), $KH_2PO4$ -0.1% (w/v) , $MgSo_4.7H_2O$-0.05 % (w/v) , $(NH_4)_2HPO_4$ -0.02 % (w/v) , Glycerol -2 % (v/v) , Peptone -0.5 % (w/v) , Yeast extract – 0.05% (w/v), pH – 7.5±0.3 (Sun *et al.,* 1994); coffee husk extract (CH)obtained from acid hydrolysis alone was used as media; CH along with 2 and 4% of glucose were also used (CH2% and CH4%) to analyze whether the organism can uptake nutrients from coffee husk and produce PHA in higher quantity.

***Extraction of PHA***

All the above media were prepared and inoculated with the bacterial isolate and incubated for 76hr. After incubation the cultures were centrifuged at 10,000rpm for 5min and pellet was collected, air dried and weighed, which gives the cell dry weight (CDW). The pellet was then treated with sodium hypochlorite (0.4% w/v) solution and incubated at 37°C for 1-2 h and again centrifuged at 10,000rpm for 5min, the supernatant was discarded and the pellet was washed with acetone and diethyl ether (1:1) and suspended in hot chloroform(Dong and Sun, 2000). The yielded PHA powder was then analyzed spectrometrically using UV Spec (Law and Spleckey,1961) which gives the concentration of PHA. To calculate the percentage of PHA content, concentration of PHA to CDW was taken and multiplied with 100 (Chien *et al.,* 2007).The experiments were performed in triplicates.

***Characterization of PHA using FT- IR analysis***

Inorder to confirm the functional groups present in PHA, the extracted PHA was added to KBr pellets and recorded in the wave number range from 400 to 4000 $cm^{-1}$ using a Perkin Elmer Fourier transform infrared (FTIR) spectrophotometer (Jasco FTIR- 6100, Japan) using KBr disc (Shamala *et al.,* 2003).

***Statistical Analysis***

All the experiments were done in triplicates and the data were analyzed statistically by standard deviation.

## RESULTS

From the serial dilution about 12 colonies were isolated and their pure cultures were screened on Nile blue agar for positive PHA producers. Among the 12 colonies of bacteria (CHDS -01 to CHDS - 12) one organism was capable of producing PHA from 24hr itself and continued till 120hr in Nile blue agar plates. Further the positive PHA producer was characterized biochemically and identified by 16SrRNA sequencing. The organism was identified as *Enterobacter aerogenes.*

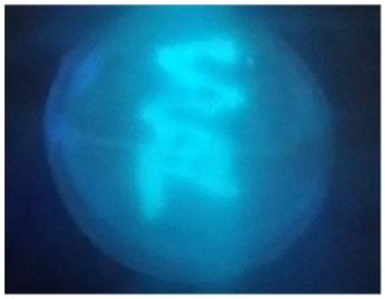

**Fig 1** Nile blue agar plate showing CHDS-03 (*Enterobacter aerogenes*) as positive PHA producer.

In order to check the organism's ability to synthesis PHA the organism was first inoculated into two types of synthetic media - A Simple Media (SM), Mineral Salt Media (MSM).

The coffee husk extract had about 38% of total sugars. The coffee husk extract was supplemented with the primarily investigated media components (SM and MSM), instead of using distilled water to these media coffee husk extract was added. CHMS produced high yield of PHA (6.4 g/L) with a CDW of 21.6 g/L (21.6%) (Table 2).

Then the organism was inoculated into coffee husk extract (CH) without supplementing it with any chemical ingredients separately and also with Different percentage of glucose (2% and 4%) without any other source of carbon or nitrogen (CH2% and CH4%). The PHA production was high at 72 hr incubation in CH2% about 7.8 g/L, CH4% 6.6 g/L and CH yielded about 5.6 g/L of PHA (Table 3). As FT-IR being a frequently used technique to analyze the molecular composition of materials, PHA extracted was subjected to FT-IR analysis. From the spectra it was evident that the organism was capable of synthesizing PHA.

**Table 1** Showing the PHA yield from Simple media (SM) and Mineral salt media (MSM) inoculated with CHDS-03 at different incubation time.

| Incubation time (hr) | SM | | | MSM | | |
|---|---|---|---|---|---|---|
| | Cell Dry weight (g/l) | PHA concentration (g/l) | % PHA (w/w) | Cell Dry weight (g/l) | PHA concentration (g/l) | % PHA (w/w) |
| 24 | 8.8±0.12 | 0.4± 0.04 | 4.54 | 9.6±0.19 | 0.4±0.05 | 4.16 |
| 48 | 12.8±0.11 | 4±0.08 | 31.25 | 10.4±0.21 | 2.4±0.02 | 23.07 |
| 72 | 15.2±0.15 | 4.8±0.06 | 31.57 | 19.6±0.24 | 6.8±0.01 | 34.69 |
| 96 | 17.4±0.16 | 4.4±0.10 | 25.28 | 12.6±0.13 | 5.6±0.07 | 44.27 |
| 120 | 11.5±0.6 | 1.5±0.01 | 13.04 | 8.4±0.10 | 3.2±0.02 | 38.09 |

Data represent the mean of 3 different readings ± standard deviation

**Table 2** Coffee husk extract along with simple media (CHSM) and MSM media (CHMSM) components inoculated with CHDS-03 at different incubation time.

| Incubation time (hr) | CHSM | | | CHMSM | | |
|---|---|---|---|---|---|---|
| | Cell Dry weight (g/l) | PHA concentration (g/l) | % PHA (w/w) | Cell Dry weight (g/l) | PHA concentration (g/l) | % PHA (w/w) |
| 24 | 7.6±0.12 | 0.10± 0.01 | 1.31 | 8.12±0.17 | 0.25±0.02 | 3.07 |
| 48 | 13.8±0.21 | 2.8±0.05 | 20.28 | 19.2±0.12 | 3.6±0.05 | 18.75 |
| 72 | 21.6±0.14 | 6.4±0.06 | 29.62 | 24.8±0.15 | 5.2±0.03 | 20.96 |
| 96 | 14.4±0.18 | 3.6±0.1 | 25 | 9.2±0.10 | 2±0.01 | 10.41 |
| 120 | 8.4±0.22 | 1.6±0.2 | 19.04 | 7.4±0.18 | 0.8±0.08 | 14.81 |

Data represent the mean of 3 different readings ± standard deviation

**Table 3** Coffee husk (CH) extract, coffee husk with 2% glucose (CH2%) and coffee husk with 4% glucose (CH4%) was inoculated with CHDS-03 at different incubation time

| Incubation time (hr) | CH | | | CH2% | | | CH4% | | |
|---|---|---|---|---|---|---|---|---|---|
| | Cell Dry weight (g/l) | PHA concentration (g/l) | % PHA (w/w) | Cell Dry weight (g/l) | PHA concentration (g/l) | % PHA (w/w) | Cell Dry weight (g/l) | PHA concentration (g/l) | % PHA (w/w) |
| 24 | 8.6±0.18 | 1.3±0.02 | 3.48 | 7.6±0.11 | 0.16±0.05 | 7.89 | 10.4±0.15 | 0.13±0.05 | 1.25 |
| 48 | 11.2±0.21 | 2.4±0.08 | 21.42 | 16.8±0.15 | 4.4±0.06 | 26.19 | 12.8±0.10 | 2.0±0.07 | 16.94 |
| 72 | 20.4±0.23 | 5.6±0.04 | 27.45 | 24.4±0.20 | 7.8±0.08 | 31.96 | 22.4±0.21 | 6.6±0.02 | 29.46 |
| 96 | 14.9±0.14 | 3.2±0.05 | 21.47 | 23.3±0.18 | 3.2±0.01 | 13.59 | 8.5±0.25 | 4.8±0.06 | 56.47 |
| 120 | 9.2±0.10 | 2.0±0.01 | 21.73 | 12.2±0.12 | 1.6±0.02 | 13.11 | 4.8±0.012 | 1.8±0.01 | 37.5 |

Data represent the mean of 3 different readings ± standard deviation

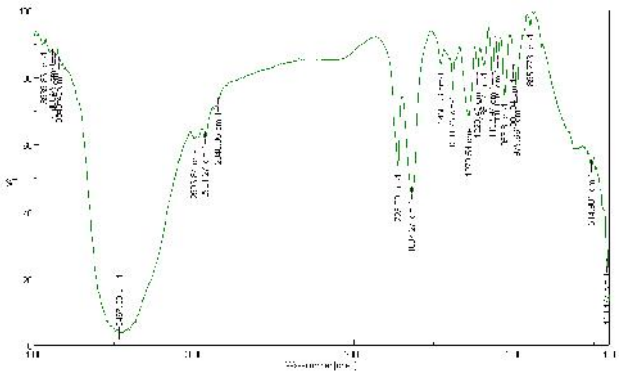

**Fig 2** FT-IR Spectra of Extracted PHA produced by *Enterobacter aerogenes.*

## DISCUSSION

Using organic material as substrate instead of synthetic ones has a myriad of benefits, hitherto cutting down half the cost for the fermentation process. The main by-products of coffee industry, pulp and husks amounts to one million tons, which remains a serious problem due to the presence of toxic materials such as caffeine, free phenols (monomers) and polyphenols (tannins) (Bressani, 1978) would serve as a cheapest substrate for PHA production. This study deals with the extensive analysis of utilizing coffee husk alone for the production of PHA and also to investigate the ability of the organism to yield higher quantity of PHA by supplementing coffee husk with simple media components.

As coffee husk extract was used as substrate it would be so appropriate to isolate PHA producers from samples related to coffee husk and hence coffee husk dumped soil was preferred. From the 12 organisms screened on Nile blue plates only one organism was selected to be the best, this was analyzed by the intensity of fluorescence exhibited by the organism on the plate and the least incubation time needed for the organism to produce PHA.

From Table 1 the results of the study yielded was about 6.8 g/L of PHA in MSM with the highest dry weight of 19.6g/L when compared to the simple media. The organism produced PHA with an accumulation rate of 44.2% in MSM. This proves the ability of the organism to produce PHA even without stress condition with the presence of all the nutrients.

However there was no drastic increase in the amount of PHA produced when coffee husk extract was supplemented with media components (Table 2). As the highest amount of PHA extracted was only 6.4g/L. But there was an increase in the amount of cell biomass in coffee husk MSM media (24.8g/L CDW) with only 20.96% PHA accumulation. Then the organism was inoculated into coffee husk extract without supplementing it with any chemical ingredients separately

(Table 3). The organism was capable of synthesizing PHA up to 120 hr where it showed the highest amount of production at 72 hr (5.6g/L PHA Concentration, 20.4g/L CDW and 27.45% PHA accumulation). The organism showed gradual increase in both biomass and PHA production up to 72hr and then it decrease interpreting that the organism has entered decline phase and began to utilize the produced PHA granules. As only minor difference was observed in the amount of PHA by organism in the synthetic media to that of the organic substrate (5.6g/L PHA Concentration (coffee husk); 6.8g/L PHA Concentration (MSM)) coffee husk extract can be successfully used as a substrate for PHA production when supplemented with simple media components.

As C:N ratio considered to be an important criteria for some PHA producers (Verlinden *et al.,* 2007) the idea of increasing the carbon content alone in the coffee husk extract was manipulated(Table 3).In this media also the organism reflected the same pattern of PHA accumulation till 120 hr with the highest point at 72 hr and decreased from 96$^{th}$ hr. It was interesting to note that coffee husk extract with 2% of glucose produced 7.8 g/L PHA with 24.4 g/L CDW and 31.96% PHA accumulation. The dry weight of PHA produced in this media was the highest when compared to all other media. In coffee husk extract with 4% glucose about 6.6 g/L of PHA (at 72 hr) was produced, it recorded the highest percentage of PHA accumulation (56.47%). This suggests that even higher amount of carbon can also hinder the synthesis of PHA. But with limited increase in carbon can have a positive impact on boosting the synthesis of PHA.

From the FT-IR spectrum it was evident that the peaks ranging from 3467.38 $cm^{-1}$correspponds to O-H stretching, 2996.84 $cm^{-1}$ C-H stretching,1723.09 $cm^{-1}$C=O representing the presence of ester carbonyl group (Hong *et al.* 1999).

## CONCLUSION

The present study focuses on PHA production from cheap source of substrate thus proving cost effective and helpful in solid waste management. Compared with that of the synthetic media, coffee husk was capable of enriching the organism to produce PHA in a better way. Hence it was clear that coffee husk supplemented with just 2% of glucose can serve as a cost effective media for the industrial level production of PHA.

## References


Bowtell D and Sambrook J. DNA Microarrays, 2002. A Molecular Cloning Manual. 1st edition. New York: Cold Spring Harbor Laboratory Press.

Braunegg G, Lefebvre G. and Genser K. F,1998. Polyhydroxyalkanoates, biopolyesters from renewable resources: Physiological and engineering aspects. *Journal of Biotechnology***,** 65, 127 - 161.

Chien CC, Chen CC, Choi MH, Kung SS, Wei YH, 2007.Production of polyhydroxybutyrate (PHB) by *Vibrio spp*. isolated from marine enviromnent. *J.Biotech.* 132, 259-263.

Hedge J.E, and Hofreiter, B.T, 1962. In: Carbohydrate Chemistry 17 ( Eds Whistler R.L. and Be Miller, J. N.), *Academic Press New York*.

Hong K, Sun S, Tian W, Chen GQ, Huang W. A rapid method for detecting bacterial polyhydroxyalkanoates in intact cells by Fourier Transform Infrared Spectroscopy. *Appl Microbiol Biotechnol*. 1999; 51: 523-526.

Law JH, Slepecky RA ,1961. Assay of poly-B-hydroxybutyric acid. *J . Bacteriol.*82, 33-36.

Lemoigne M ,1976. Products of dehydration and of polymerization of β-hydroxybutyric acid. *Bull Soc Chem Biol* ,8, 770-82.

Narasimha Murthy K.V, Antonette D'Sa and Gaurav Kapur. An effluent treatment-cum-electricity generation option at coffee estates: is it financially feasible? , International Energy Initiative, Bangalore.

Shamala TR, Chandrashekar A, Vijayendra SVN, Kshama L, 2003. Identification of polyhydroxyalkanoate (PHA)-producing Bacillus spp. using the polymerase chain reaction (PCR). *J. App. Microbiol*. 94, 369-374.

Shang L, Jiang M and Chang H. N, 2003. Poly (3-hydroxybutyrate) synthesis in fed-batch culture of Ralstonia *eutropha* with phosphate limitation under different glucose concentrations. *Biotechnology Letters*. **25**, 1415 - 1419.

Spiekermann, P, Rehm, B.H.A, Kalscheuer, R, Baumeister, D, Steinbuchel, A, 1999. A sensitive, -colony staining method using Nile red for direct screening of bacteria that accumulate polyhydroxyalkanoic acids and other lipid storage compounds, *Arch. Microbiol.* 171, 73-80.

Subbiah Poopathi and S. Abidha 2011. Coffee Husk Waste for Fermentation Production of Mosquitocidal Bacteria. *Journal of Economic Entomology*. 104(6):1816-1823.

Sun W, Cao JG, Teng K, Meighen EA, 1994. Biosynthesis of poly-3hydroxybutyrate in the luminescent bacterium, Vibrio harveyi, and regulation by the lux autoinducer 'N-(3-hydroxybutanoyl) homoserine lactone, *J. Biol. Chem*. 269, 20785-20790.

Verlinden R.A, Hill D.J, Kenward M.A, Williams C.D, Radecka I, 2007. Bacterial synthesis of biodegradable polyhydroxyalkanoates, *J Appl Microbiol*, 102, 1437-1449.


**How to cite this article:**
K. Preethi *et al*., Bioremediation Of Coffee Husk Through Polyhydroxyalkanoates (Pha) Production For A Greener Environment. *International Journal of Recent Scientific Research Vol. 6, Issue, 6, pp.4857-4860, June, 2015*

*******