Advances in Bioresearch
Adv. Biores., Vol 9 (4) July 2018: 167-169
©2018 Society of Education, India
Print ISSN 0976-4585; Online ISSN 2277-1573
Journal's URL:http://www.soeagra.com/abr.html
CODEN: ABRDC3
DOI: 10.15515/abr.0976-4585.9.4.167169

Advances in Bioresearch

SHORT COMMUNICATION

Pretreated Paddy Straw Hydrolysate Based Medium For Production of *Azotobacter* Biofertilizer

[1]Seema Garcha and [2]Rupsi Kansal*
Affiliation-[1,2]Department of Microbiology, Punjab Agricultural University, Ludhiana 141004, India.
*Corresponding author email- rupsi16kansal@gmail.com

ABSTRACT
An effort was made to determine utility of paddy straw hydrolysate based natural medium for biomass production of Azotobacter, a free living nitrogen fixing bacteria. Paddy straw is one of the most abundant lignocellulosic waste that mainly consists of cellulose, hemicellulose, lignin and water soluble polysaccharides. Pulverized paddy straw was pretreated with 2% KOH for delignification followed by two phased acid hydrolysis coupled with high temperature treatment. A cell biomass of 2 x 10^5 cfu/ml was harvested from supplemented paddy straw after ten days of incubation at 28^{0}C/120 rpm which was 66.7% of the biomass obtained using synthetic Jensen's medium. 70.4% of total fermentable reducing sugars were utilized by the microbe.
Keywords: Azotobacter, biofertilizer, biomass generation, paddy straw hydrolysate.

Received 15.02.2018 Revised 19.05.2018 Accepted 16.06.2018

How to cite this article
Seema Garcha and Rupsi Kansal. Pretreated Paddy Straw Hydrolysate Based Medium For Production Of *Azotobacter* Biofertilizer. Adv. Biores., Vol 9 [4] July 2018.167-169

INTRODUCTION

Biofertilizers are environment friendly alternative to chemical fertilizers. They enrich soil microflora and contribute towards sustainability of agriculture. *Azotobacter* is a free living nitrogen fixing bacteria. It helps to provide nitrogen to the plant in usable form and also helps to promote plant growth [1]. A high dose of micro-organisms is to be cultivated under suitable conditions for biofertilizer production. Cost-effective bacterial biomass generation is very crucial for viability of this task. Usually, a count of nearly one billion cells per unit of medium should be applied as biofertilizer to obtain the desired benefit [2]. Microbial biomass generation is generally carried out using semi-synthetic/synthetic microbiological media which forms a major expanse of this activity.

Paddy straw is one of the most abundant lignocellulosic waste generated. Main constituents of paddy straw are cellulose 38-41%, hemicelluloses 26-35%, lignin 15% and 8% water soluble polysaccharides [3]. Pretreated paddy straw can be utilized as substrate for microbial fermentation by many bacteria and fungi including *Azotobacter* spp [4,5]

MATERIAL AND METHODS

Pretreated paddy straw was prepared as per the method of Yadav *et al* [2]. 75g of paddy straw was delignified with 0.2M KOH at 10% level. Potassium hydroxide was used for alkaline treatment instead of sodium hydroxide [6]. It was kept at room temperature for 4h. After that, it was filtered with muslin cloth and the residue was washed with the help of water until pH reached near neutral. Residue was then dried till constant weight. Two phased acidic hydrolysis was then performed at 1:10 w/v solid to liquid ratio.

In the first step, 1% sulphuric acid was used for acid hydrolysis followed by autoclaving (121^{0}C, 15 psi) for 45 minutes. Second stage acid hydrolysis was carried out with 2% sulphuric acid and autoclaving (130^{0}C, 23 PSI) for 60 minutes. Calcium oxide was added to the mixed hydrolysate with continuous stirring until the pH reached 10.0. After incubating it for half an hour, it was centrifuged at 3000g for 20 minutes and filtered out. pH was adjusted to 6.0. To remove furans, 3.5% w/v of activated charcoal was added to the hydrolysate and it was placed on shaker for stirring for 1h. Centrifugation at 3000g for 20

minutes was repeated and filtration was done. Fermentable reducing sugars and furans were estimated by the method of DNS and spectroscopic analysis, respectively[7,8]. The hydrolysate was used for bacterial biomass generation.

As per the nutritional requirement of *Azotobacter*, hydrolysate was supplemented with $CaCO_3$ (2.0gm/l), K_2HPO_4 (1.0gm/l), $MgSO_4$ (0.5 gm/l), NaCl (0.5gm/l), $FeSO_4.7H_2O$ (0.1gm/l), Na_2MoO_4 (0.005gm/l). TSS content was recorded using Brix refractometer. 7% v/v of metabolically active culture of *Azotobacter* containing 2 X 10^7 cfu/ml was inoculated in the hydrolysate. Incubation of $28^0C \pm 2^0C$ at 120rpm was provided. Standard microbiological procedures were followed to determine cell biomass increase in the sample run and compared to the cell biomass generated in recommended Jensen's medium. Amount of fermentable reducing sugars and other parameters were recorded pre and post incubation.

RESULTS AND DISCUSSION:

Pulverized paddy straw was found to contain 42% ADF (acid detergent fibre) and 59% NDF (neutral detergent fibre). As widely reported, the presence of lignin interferes with saccharification of polymers into fermentable monomeric sugars. Alkaline pretreatment saponifies intermolecular ester bonds, cross linking hemicellulose and lignin.

The DNS content after delignification and acid hydrolysis, (which was carried out for depolymerization of cellulose and hemicellulose) was estimated to be 18.25mg/g. Furans were reduced from an initial of 0.714mg/g after delignification to a final of 0.071mg/g after treatment with activated charcoal, a reduction of 90.5%. Appearance of paddy straw hydrolysate changed from a dark coloured to a colourless turbid liquid. After inoculation with *Azotobacter*, it was observed for the signs of microbial growth. After nearly ten days a cell biomass of 2 X 10^5 cfu/ml was obtained compared to 3 X 10^5 cfu/ml in the Jensen's medium, shortfall of 33.3%. DNS was recorded at 5.04 mg/g, 70.4% of total fermentable reducing sugars were utilized. Post incubation the pH had risen to 7.2 from an initial pH of 6.0.

CONCLUSION

Paddy straw hydrolysate is a good source of fermentable sugars. It can act as a natural growth medium for biomass generation of biofertilizers aka *Azotobacter*. Removal of furans facilitated bacterial growth. 2 X 10^5 cfu/ml of *Azotobacter* cell count was obtained in supplemented paddy straw hydrolysate which is 66.7% of the growth obtained in semi synthetic Jensen's medium. Process optimization using statistical software is underway. Pilot scale studies will be carried out subsequently.

ACKNOWLEDGEMENTS

Funding provided by the Directorate of Research, Punjab Agricultural University, Ludhiana and the laboratory facilities extended by the Department of Microbiology and School of Renewable Energy and Engineering are gratefully acknowledged.

COMPETING INTEREST

The authors have declared that no competing interest exists.

REFERENCES

1. Singh, A. K., Gauri, Bhatt, R. P. & Pant, S. (2011). Optimization and comparative study of the sugar waste for the growth of *Rhizobium* cells along with traditional laboratory media. Res J Microbiol.,6:715-723.
2. Yadav, A. K. (2011). Production Technology for biofertilizer, Organic Fertilizer and other Organic Inputs, National Centre of Organic Farming, Department of Agriculture and Cooperation, Ministry of Agriculture, Gol, Ghaziabad, pp 5.
3. Weber, S., Stubner, S. & Conrad, R. (2001). Bacterial populations colonizing and degrading paddy straw in anoxic paddy soil. Appl Environ Microbiol.,67:1318-1327.
4. Albareda, M., Rodriguez-Navarro, D. N., Camacho, M. & Temprano, F. J. (2008). Alternatives to peat as a carrier for rhizobia inoculants: solid and liquid formulations. Soil Biol Biochem.,40:2771-2779.
5. Mosier, N., Wyman, C., Dale, B., Elander, R., Lee, Y. Y., Holtzapple, M., & Ladisch, M. (2005). Features of promising technologies for pretreatment of lignocellulosic biomass. Bioresour Technol., 96:673-686.
6. Curling, S. (2005). Timber residue as a source for hemicellulose process chemicals. Retrieved from http:// www.rrbconference.ugent.be/presentations/Curling{%}20Simon.pdf
7. Martinez, A. Rodriguez, M. E., York, S.W., Preston, J.E, Ingram, & L.O. (2000). Effects of Ca $(OH)_2$ treatments ("overliming") on the composition and toxicity of bagasse hemicellulose hydrolysates. Biotechnol Bioeng., 69:526–536.

8. Miller, G.L. (1959). Use of dinitrosalicylic acid reagent for determination of reducing sugar. Anal Chem., 31:426–428.