GLOBAL JOURNAL OF BIO-SCIENCE AND BIOTECHNOLOGY

www.scienceandnature.org

A COMPARATIVE STUDY OF ROTTEN PINEAPPLE PEELS AND OTHER SOURCES FOR THE PRODUCTION OF SINGLE CELL PROTEIN

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ABSTRACT
Research on single cell proteins to cope with the problem of worldwide food scarcity has been progressing fast for a couple of years. New technologies are being developed to reduce the cost and to enhance the quality of the product. Present study focuses on the comparative analysis and includes isolation of yeast, inoculums development, collection of waste, and biomass and protein estimation. Turbidity due to growth of yeast cells was checked in media and found the highest in the GSFH. This media shows better results than others in the study. Wet and dry masses were recorded as 29.8 g/l and 12.6 g/l respectively. Protein concentration was obtained 0.6 g/g of dry biomass in 500 ml of media. So in all experiments our media gave the best results and can be used for the production of single cell proteins in the cost effective manner.

KEY WORDS: Rotten pineapple, Yeast, Single cell proteins, GSFH.

INTRODUCTION

Every ninth person of the world was suffering from hunger as estimated by the United Nations Food and Agriculture Organization in 2014-15 and developing countries are the most affected. However several policies by governments have been framed and implemented. Around 3.5 million children sleep forever annually due to undernourishment in the world. One of root causes is that people do not have money to access the food (Saquido *et al*., 1981). So if approaches to reduce the cost on food are adopted and technologies devised for the same these higher death rates could be decelerated to great extent. Researchers of different fields are trying hard and biotechnologists have greater share in alleviating this problem. Production of single cell proteins with cost effective technology may be a good choice. Carrol L. Wison gave the term SCP in 1966. SCPs comprise dry, dead microbial cells or extracted proteins. These can be used as supplementary food. SCPs production is possible with lesser size of land for the large quantity of produce than that for the traditional crops and comparing the two types of produces traditional crops are costlier. SCPs production is independent of seasonal factors (Abou Hamed *et al*., 1993). Another reason supporting the production of SCPs is their involvement in the reduction of environmental pollution as SCPs can be produced through bioconversion of the waste. However algae are also capable of producing single cell proteins but their physical and chemical requirements are much more, moreover those are difficult to digest. Waste from food processing industry is a raw material for the production of SCPs. Main microbes which convert waste into SCPs are yeasts. Rotten pineapple and its peel can be used as raw material which is important fruit of India and cultivated in 89 thousand ha. It is grown mainly in Karnataka, Kerala, Maharashtra and West Bengal in India. As per Indian Horticulture Database 2011 area for pineapple production has increased from 87 thousand ha in 2006-07 to 89 thousand ha in 2010-11 (Bacha *et al*., 2011). Different varieties like kew, common queen and Mauritius are grown in India. As per Indian Agricultural Research Data book 2004, 50 million tonnes of vegetables and fruits are wasted annually (Fawcett *et al*., 1960). Microorganisms can utilize a variety of substances like agricultural waste, industrial effluents, natural gas like methane *etc*. and help in decomposing pollutants (Hansen *et al*., 1975). SCPs are produced by many types of microorganisms like Yeast, fungi, bacteria, algae. *Saccharomyces cerevisiae*, *Geotrichum candidum* and *Candida utilis* are some yeast which produces SCPs. In fungi *Aspergillus fusarium*, *Sclerotium* is capable of producing SCPs (Karla *et al*., 1989). SCPs can be grown even on effluents of biogas. Towards the approach of SCPs production prepared media containing the rotten pineapple extract was used, inoculums developed and single cell proteins were obtained after the fermentation process. Cell count study, pH determination, biomass and protein estimation were also performed. In the section of results and discussions a comparative study is emphasized.

MATERIALS & METHODS

Isolation of yeast

Rotten fruit was selected as a source for isolation of yeast. Rotten Pineapple's pulp as well as skin was taken, crushed and filtered. From 10^{-1}, 10^{-2} and 10^{-3}dilutions 0.1ml of the filtrate was spread on GYE plates. Plates were then incubated at room temperature in incubator for 48-72 hours. After incubation period morphological characters were studied.

Inoculum development

100 ml of GYE medium was prepared in the flask previously sterilized in autoclave. Well isolated colonies were studied and selected for inoculation. Pure Colonies were picked up with the help of a sterile wire loop and inoculated into the medium. Then the flask, along with the control was kept on shaking condition on rotary shaker for 72 hours at 120rpm.

Collection of waste

Peels of pineapple and pomegranate were taken and dried for almost 7 days. After 7 days peels were cut into small pieces. These small pieces were oven dried at 55˚C in Hot air oven then crushed to make a fine powder containing mixture of both the peels.

Preparation of media

Three media each having different formulations were prepared. Those three media were

GSFH- Glucose supplemented fruit hydrolysate medium

SFH- Supplemented fruit hydrolysate medium

FHM- Fruit hydrolysate medium.

All the components listed below were carefully weighed on high precision balance.

The compositions of all the three media (100 ml) differ from one another as shown below.

	GSFH	SFH	FHM
Fruit powder	1.0g	1.0g	1.0g
D-glucose	1.0g	-	-
$(NH_4)_2SO_4$	0.5g	0.5g	-
KH_2PO_4	0.1g	0.1g	-
$MgSo_4 \cdot 7H_2O$	0.05g	0.05g	-
NaCl	0.01g	0.01g	-
$CaCl_2$	0.01g	0.01g	-

1 ml of the inoculum was inoculated into each of the three flasks having 150 ml media and also in the flask of GYE medium (containing the same amount of media) and was kept on shaker for 72 hours. This was also done in order to let organism adapt to the media conditions and to check whether the prepared media serve as a proper nutritional source to the organism or not and cell counting was done at the end of 72 hours (Krishnaveni *et al*., 1984).

Fermentation of single cell protein:

Three media, other than control were prepared as per composition listed above. The media were distributed in flasks and sterilized at 121°C for 15minutes.The inoculums of yeast culture were inoculated in the media and incubated at 28°C for 7days.

Cell count study

Cell count study was done after 72 hrs. of fermentation. Due to heavy microbial turbidity, a dilution of 1:50 was taken. Cell counting was done with the help of Neubauer's chamber. Dilution was prepared by taking 0.1mL of the sample and 4.9mL of the distilled water. After preparing the dilution the required amount was carefully loaded onto the hemocytometer. After loading the sample, it was kept in an undisturbed condition for some time and then observed under 40x lens of the microscope.

Determination of Yeast Biomass

Dry and wet biomass was determined by using centrifuge. The fermentation broth was centrifuged at 10,000 rpm at for 5 minutes from each flask. The supernatant was discarded and the cell pellets was carefully scraped on to a whatman filter paper No.1 and the difference of weight was calculated. For the measurement of dry biomass, same filter paper was kept in Hot air oven for 45˚C for 24 hours. After 24 hours, weights of the filter papers were noted again and the difference was calculated in order to get the results of dry biomass (Prosser *et al*., 1991).

Protein estimation:

The yeast cells were separated by washing from the fermentation broth and the protein content was checked by using Folin-Lowry method (Lowry *et.al*., 1951).

RESULTS & DISCUSSION

Morphological Characterization

Morphological and colonial characteristics of the colonies plated on GYE medium are as mentioned in Table 1 and Table 2 respectively.

TABLE 1: Colonial Characterization of yeast

Characters	Observation
Size	Intermediate
Shape	Round
Elevation	Flat
Texture	Smooth
Margin	Entire
Opacity	Translucent
Pigment	Nil
Consistency	Moist

TABLE 2: Morphological Characterization of yeast

Size	Shape	Arrangement	Gram Reaction
Big	Oval	Singly, budding, in cluster	Positive

FIGURE 1: Colonies of Yeast on GYE agar plate

Turbidity observation

The media in the flask kept on shaker after the inoculation of isolated colonies at 120 rpm for 72 hours developed good amount of turbidity, due to the growth of the yeast cells in it. Maximum biomass of yeast was recorded with 2% inoculum size. Duration of inoculum also matters as the highest amount of biomass was recorded when 72 hours old culture is inoculated in fermentation media.

Cell count study

The comparative study of Cell biomass in fermentation media done in order to compare the prepared media with the synthetic media; GYE shows following results (Table 3).

TABLE 3: Comparative study of Cell Count in synthetic media and prepared media

Name Medium	Cell count (cells/ml)
GSFH	46.25×10^7
SFH	34.25×10^7
FHM	37.50×10^7
Synthetic media(GYE)	45.75×10^7

As the media having formulation of GSFH showed maximum results in terms of number of cell counts considered for further study. Maximum turbidity was obtained in GSFH and maximum crude protein recorded after 8 days of incubation. Maximum SCP production was observed when glucose was added to the suppelemented fruit hydrolysate medium.

pH determination

There was decrease in pH gradually with the time as the sugars were utilized by the organism and converted to organic acids which results in the falling of pH (Figure 1).

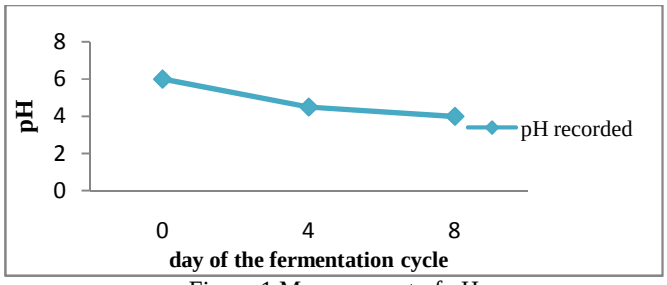

Figure 1 Measurement of pH

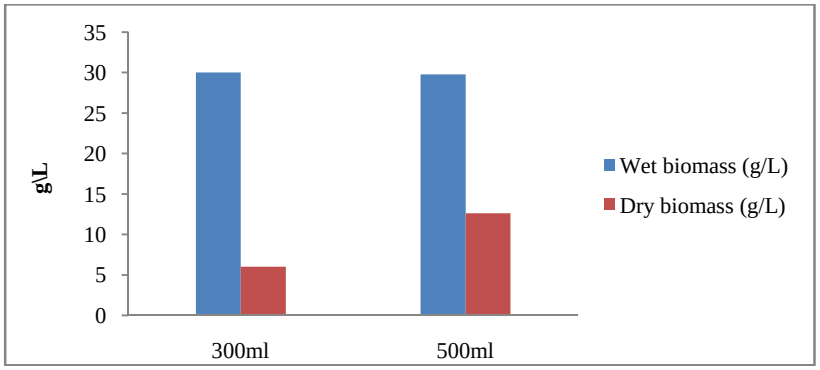

FIGURE 2: Biomass Determination

Biomass Determination

wet and dry biomass of yeast were determined (Table 4). Pine apple peels have ample amount of minerals which increases the growth of biomass (Rashad *et al*., 1990).

Protein estimation

Protein estimation was performed by the Folin-lowry's method (Lowry *et al*., 1951) (Figure 3). The results of Protein estimation are mentioned in g/l to compare with the biomass concentration obtained.

TABLE 4: Biomass estimation

Media (Qty)	Wet biomass (g/L)	Dry biomass (g/L)
300mL	30	6
500mL	29.8	12.6

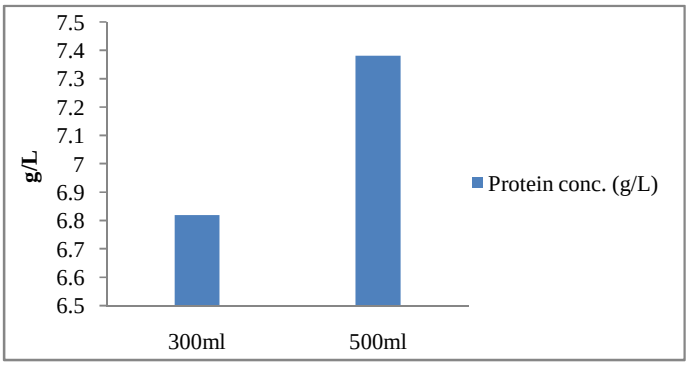

FIGURE 4: Estimation of protein

CONCLUSION

The Production of single cell proteins using yeast with different media was assessed. The amount of biomass and single cell proteins were found highest with glucose supplemented fruit hydrolysate media (GSFH) compared to Synthetic media. High turbidity was developed due to growth of yeast cells in 72 hours of incubation. GSFH media was found a good option for the production of single cell proteins and in future it can be more appropriately formulated with deliberation.

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