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Research Article

Conversion of food processing wastes in to valuable by – products

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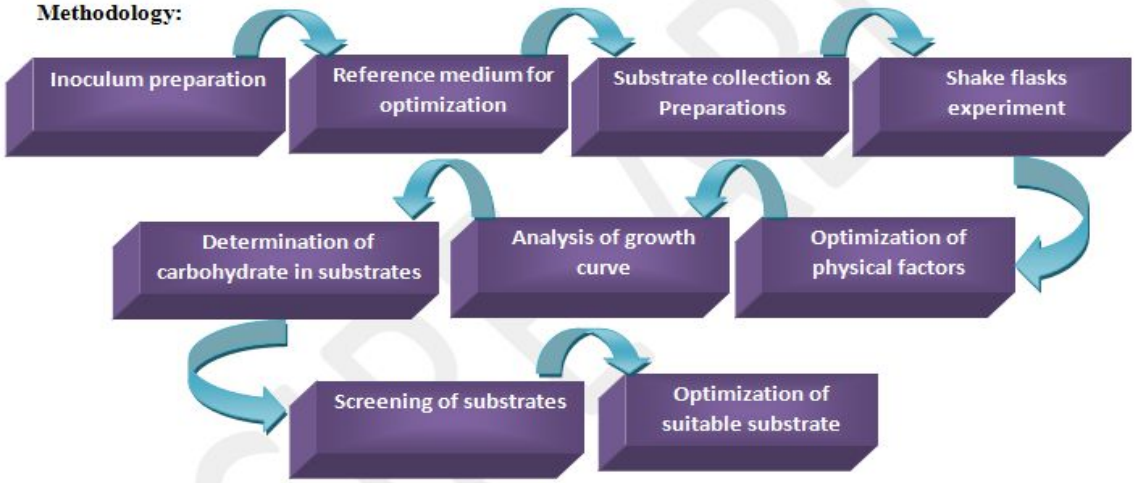
Objective: Agricultural by-products are excellent sources of energy nutrients. Screening & utilization of these low cost by-products as medium to generate single cell proteins which have potential application in food industry

Methodology:



Duration taken for the research: 6 months

Conclusion: The present study concluded that food processing waste product of jack fruit pulp selected from other substrates such as pulp of mango, water melon, maize and tamarind used as substrates to generation of yeast biomass as single cell protein. The efficient utilization of the food processing waste-products from fruit pulp can help in reducing the negative cost, reduce environmental pollution, demonstrating sustainability in food industry and that has direct impact on the economy of the country.

Applicable Industries: Feed, food, dairy, cosmetics and pharmaceutical industries

Expected outcome: Low cost single cell protein (SCP) production by using food processed by-products

Scale up hurdles, if any: Generation of yeast biomass in laboratory scale and pilot scale fermentors

Abstract

Single-Cell Protein (SCP) represents microbial cells (primary) grown in mass culture and harvested for use as protein sources in foods or animal feeds. In the present study, mainly focused on scale up production of yeast biomass was using different cheap carbon source such as mango, cashew apple, jackfruit, apple, maize, watermelon, tamarind, guava and sweet potato pulps. Determination of carbohydrate content of each substrate by anthrone method was maximum at jackfruit (1.45mg/ml). For the scale up production the primary screening of the fruit pulps by shake flasks experiment. Optimization of different physical factors and carbon sources has been done to find out the range and levels.

Keywords: Yeast, Substrates, fruits, Single cell protein, optimization, Shake flask

Introduction

In recent years increasing attention has been given to the conversion of industrial by-products into valuable products such as Single Cell Protein (SCP). SCP production is an efficient way of converting any waste carbohydrate into protein-rich feedstuff. The recovery of value added products and the simultaneous reduction of the organic load of the waste are the chief advantages of such processes. Generation of protein rich biomass from a waste that would have added to the cost of disposal and thereby earning carbon credits is always an attraction in industrial sector (Nigam and Kakati, 2002).

The development of an economically viable culture medium is necessary to produce yeast biomass on an industrial scale. The constituents of a medium must satisfy the basic requirements for cell biomass and metabolite production, by providing an adequate supply of energy for biosynthesis and cell maintenance (Costa *et al.*, 2002). Designing the fermentation media is of critical importance because the media composition can significantly affect product concentration, yield, and volumetric productivity and ease the cost of downstream product separation (Baishan *et al.*, 2003).

The raw materials used as substrate for industrial yeast biomass production are usually sorghum hydrolysate, sulfate waste liquor, prawn-shell wastes, dairy wastes, molasses stillage, starch, plant origin liquid waste, forestry and food waste by-products. *Candida utilis* proved its potential to utilize sulphite waste liquor from the paper pulping industry as the sole carbon source (Litchfield, 1979). Crude media are more likely to provide excess of toxic ions than to be deficient in required ions. Several of the better crude

nutritive sources are in themselves complex mixtures of nutrients, supplying carbon and nitrogen compounds as well as microbial growth factors (Casida, 1968). Ornelas-Vale *et al*. (1977) succeeded in producing single cell protein from the nopal fruit using *C. utilis*. Single cell protein production from mandrin orange peel was studied using various yeast strains like *S. cerevisiae*, *C. utilis*, *Debaryomyces hansenii* and *Rhodotorula glutinis* by Nishio and Nagai (1981). Single cell protein production from *Schwanniomyces castellii* was optimized using cassava starch as carbon source (Hongpattarakere and Kittikun, 1995). Konlani (1996) optimized the cell yield of *Candida krusei* SOI and *Saccharomyces sp.* LK3G cultured in sorghum hydrolysate. Ram horn hydrolysate was used as carbon source for the single cell production of *C. utilis*, a significant waste product of the meat industry in Turkey (Kurbanoglu, 2001). Marine yeast *Cryptococcus aureus* G7a was suitable for single cell protein production from Jerusalem artichoke extract used as carbon source (Lingmei *et al*., 2007). Economic production of single cell protein from yeast *Debaryomyces hansenii*, *Kluyveromyces marxianus* and *Pichia stipitis* using non -detoxified breweries and spent grains hemicellulosic hydrolysate have been reported (Duarte *et al*., 2008).

The recovery of such by-products can significantly reduce the costs of waste disposal, and SCP generation promises wide scale application in various areas, particularly as a feed supplement. The usage of such wastes as the sole carbon and nitrogen sources for the production of SCP by microorganisms could be simply attributed to their abundance and low cost. In this way the present study mainly focused on generation of SCP from fruit waters.

Materials and methodology

Yeast strain

Yeast strain was cultured in malt extract medium in distilled water with malt extract 17gL^{-1}, mycological peptone 3gL^{-1} and agar 20gL^{-1}.

Preparation of inoculum

Mineral based medium was solidified with 2% agar and made into slants. The yeast isolate was streaked on to slants and incubated at 26 ± 1°C for 48 hours. The cells were harvested at the logarithmic phase using 0.5% sterile saline. Optical density of the culture suspension was taken at 540nm in a UV-Vis spectrophotometer (Shimadzu UV-1601) and absorbance was adjusted to 0.1 by appropriate dilution and 0.1% of this suspension was used as the inoculum.

Selection of medium for optimization

Culture medium reported by Tobajas *et al.* (2003) which was considered to be the best for biomass production of yeast was selected in this experiment for optimization. Mineral based medium had the following composition (gL^{-1}): D-glucose - 10; $(NH_4)_2SO_4$ -5; KH_2PO_4 -5; $MgSO_4.7H_2O$ - 0.5; $CaCl_2$ - 0.13; yeast extract - 0.5.

Substrate preparation

Fruits such as pine apple, cashew apple, maize, mango, jack fruit, tamarind, apple, sweet potato and guava were mashed using a mortar and pestle and centrifuged. Supernatant of samples were collected and sterilized separately (10lbm/10min) which were add into sterilized mineral based medium.

Optimization of physical factors

Shake flask experiment

The optimization of growth conditions was carried out in Erlenmeyer flasks (250ml) with malt extract broth.

Optimization of NaCl content

Malt extract broth was prepared aliquots at different salinities such as 0, 10, 20, 30, 40, 50, 60, 70, 80, 90 and 100 by addition of NaCl (gL^{-1}) to distilled water. The flasks were inoculated with 0.1% cell suspension having an absorbance of 0.1 at Abs_{540} nm and incubated at 28±1°C for 72 hours on a rotary shaker at 120 rpm. Growth was measured as absorbance at 540 nm using a UV- Vis spectrophotometer (UV-1601, Shimadzu Corporation, Tokyo, Japan).

Optimization of pH

Malt extract broth was prepared in aliquots in saline water (20 gL^{-1}) optimized from the previous experiment. pH was adjusted to 3, 3.5, 4, 4.5, 5, 5.5, 6, 6.5, 7, 7.5, 8 and 8.5 using 1N HCl and 1N NaOH, inoculated and incubated, and growth measured as detailed earlier.

Optimization of Temperature

Malt extract broth prepared in aliquots in saline water (20 gL^{-1}) having pH adjusted to 4.5 was inoculated with yeast suspension as described previously. The flasks were incubated at different temperatures such

as 20, 25, 30, 35, and 40ºC in a temperature controlled rotary shaker (Orbitek - Scigenics Biotech (Pvt) Ltd, Chennai, India) at 120 rpm. Growth was measured absorbance at 540_{nm} using spectrophotometer.

Analysis of growth at different time intervals

Samples were aseptically withdrawn from the flasks at 24 hours intervals. Cells were separated by centrifugation at 7500 x g for 10 min at 4°C. The pellets were repeatedly washed in sterile saline ($5gL^{-1}$ NaCl), re-suspended in fresh saline and measured absorbance at 540nm in a UV-Vis spectrophotometer and converted to dry cell mass using a standard curve constructed as described by Guerra and Pastrana (2002).

Optimization of carbon source

Total Carbohydrates

Total carbohydrate in the substrates was determined spectrophotometrically by Anthrone method (Hedge and Hofreiter, 1962). Quantities of 0 to $100\mu gmL^{-1}$ concentrations of glucose were used to plot a standard curve and from this curve, concentration of carbohydrate was calculated. Amount of carbohydrate was expressed as dry weight (gL^{-1}).

Carbon source was varied in the first set of experiments. Different carbon sources used were Pine apple, Cashew apple, maize, Mango, Jack fruit, tamarind, apple, sweet potato and guava. Glucose used as control. The flasks were inoculated with 0.1% cell suspension and incubated at 26.3°C for 72 hours on a rotary shaker at 100rpm and growth was measured from the absorbance at Abs_{540nm} in a spectrophotometer (UV-1601, Shimadzu Corporation, Tokyo, Japan).

Results and discussions

The cell suspension having absorbance at Abs_{540nm} ($5.57x10^5$cfu mL^{-1}) was chosen as the inoculum size for further studies.

Shake flask experiment

Optimization of physical factors

The selection of optimum range of each medium component for obtaining maximum biomass was carried out in this set of experiments.Under one dimensional screening to find out the optimum range of each

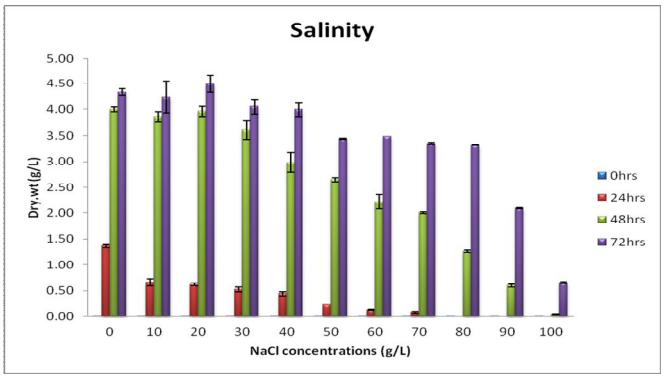
parameter such as NaCl content, pH, and temperature, regression analysis was performed (Table 1) and wherever found significant, the corresponding range was accepted for further analysis. Accordingly, NaCl content 0-50gL^{-1} (Figure 1a), pH 3.5-7.5 (Figure 1b), and temperature 20-35ºC (Figure1c) were the optimum ranges. Physical parameters viz., salinity (NaCl concentration), pH and temperature are the variables addressed in preliminary experiments considering the design and economy of fermentation process (Sheng *et al*., 2009). Yeasts are known to grow over a broad pH range from 2 to 9 (Lal et al., 2009). Anas and Singh (2003) reported that the yeast *Acremonium diospyri* preferred pH 4 for higher cell yield.

The preliminary experiment was one dimensional screening (initial screening experiment) of growth conditions in order to find the significant range of physical factors affecting the biomass production.

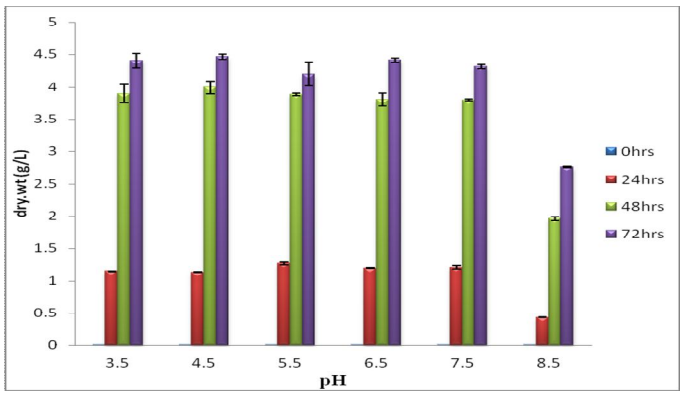
The yeast was capable of growing in a range of 0 -100gL^{-1} concentration of NaCl in shake flask experiments. The growth exponentially increased with the concentration of NaCl from 0 - 40gL^{-1} but the production slightly decreased at concentrations 50-80gL^{-1} NaCl and noticeably decreased from 80-100gL^{-1}. Maximum biomass was obtained at the concentration of 20gL^{-1}. In the case of pH it was observed that the biomass production was possible within the range of pH 3.5-7.5 and the maximum was obtained in pH 4.5. Yeast grew well at temperature in the range 20-35^{o}C and maximum production was observed at 25^{o}C. This screening experiment considered the significance of one factor at a time and did not take into account, the interaction of physical parameters as a whole in the biomass production.

Table 1 Experimental range and levels of the independent variables

Variables	**Code**	**Range studied**	**Range and levels**		
			-1	0	1
Salinity (gL^{-1})	A	0-50	0	25	50
pH	B	3-7.5	3.5	5.5	7.5
Temperature(ºC)	C	20-35	20	27.5	35



(a)



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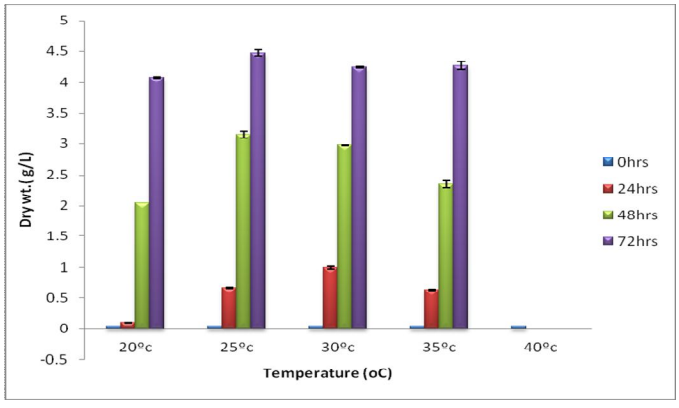

(c)

Figure 1 (a-c): One dimensional screening of physical factors such as NaCl, pH and temperature affecting biomass production

Table 2: Yeast biomass generation in different raw fruits

Fruits	Biomass yield (g-1)
Jackfruit	1.45
Tamarind	0.525
Sweet potato	0.49
Cashew apple	0.46
Guava	0.42
Apple	0.36
Maize	0.34
Watermelon	0.27
Mango	0.22

Analysis of growth at different time intervals

Absorbance of 1.0 optical density (O.D) of yeast cells in suspension (wet weight) corresponds to 0.4669gL^{-1} dry weight.

Optimization of carbon source

Total carbohydrate content of raw fruits such as jackfruit, tamarind, sweet potato, cashew apple, guava, apple, maize, watermelon and mango were observed (Table.2). It was observed that jackfruit, tamarind, maize, watermelon and mango are having highest amount of carbohydrate content and so they were selected for the further optimization experiments. Result obtained in the present study suggested that the optimum production of biomass in the carbon was jackfruit (Figure 2).

In biotechnology-based industrial processes, the formulation of cultivation media is of critical importance as their composition affects product concentration, yield and volumetric productivity. It is also important to reduce the costs of the medium as this may affect the overall process economics (Souza *et al.*, 2006). The industrial production of SCP is greatly affected by the cost of the medium and substrate used, among which the substrate costs are the largest single cost factor involved. In this context the present

study developing an appropriate medium with jack fruit which can significantly save production cost. Simplification of the manufacture and purification of the raw material can save the costs.

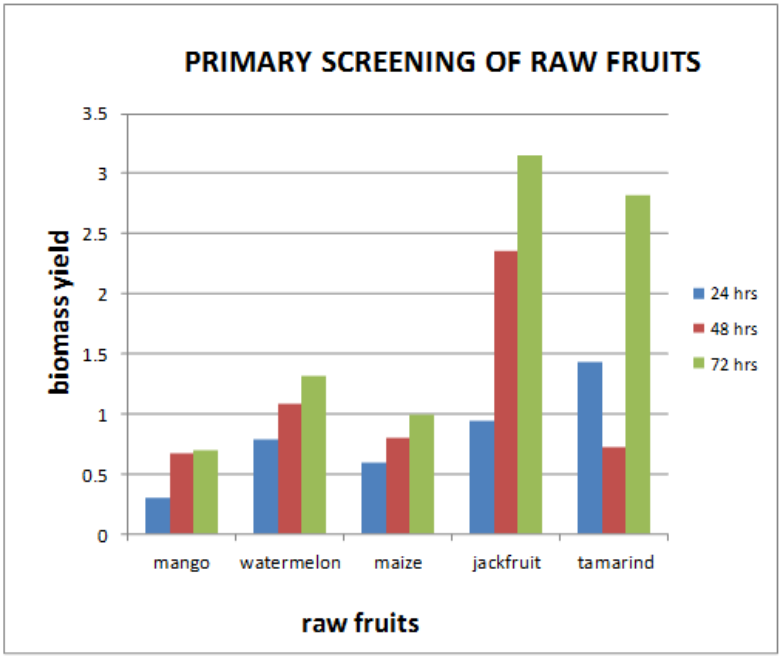

Figure 2: Biomass generation in different raw fruits

Conclusion

The present study concluded that generation of yeast biomass in raw fruits used as substrate brings it to increase the attention as valuable product. The cell suspension having absorbance 0.1 at Abs_{540nm} ($5.57x10^5$cfu mL^{-1}) was chosen as the inoculum size. Accordingly, NaCl content 0-50gL^{-1}, pH 3.5-7.5, and temperature 20-35ºC were the optimum ranges. Absorbance of 1 of yeast cells in suspension (wet weight) corresponds to 0.4669gL^{-1} dry weight. The optimum production of biomass in the carbon source was jackfruit.Over all study of experiment bring improve the scale up production of the desired product. This leads to reduce by the costs involved in production and increase the demand by the quality of the product.

Social relevance and expected outcome

Social relevance of the present study is generation of SCP using different raw fruits which is the byproduct of the fruit processed industries. Developing an appropriate medium with jack fruit constituents optimized can significantly save production cost.

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