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APPLICATION OF CITRUS (ORANGE) PEELS FOR PRODUCTION OF SINGLE CELL PROTEIN

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ABSTRACT:

The expansion of citrus processing plants and development in citrus technology brought about problems of disposal of the waste remaining after extraction of juice or making other products from fruit. Recycling of wastes has not only become as necessity but also a challenge in these days of energy crisis. In the present work attempts had been made for the production of single cell protein in the form of microbial biomass utilizing agricultural waste i.e. orange peels under laboratory conditions. Bacterium *Pseudomonas fluorescence*, fungus *Aspergillus oryzae* and yeast *Saccharomyces cerevisiae* were used for checking their ability to grow on citrus waste. *Saccharomyces cerevisiae* gave maximum biomass so, utilized for further study. 10% concentration of citrus waste gave maximum production of biomass under submerged conditions i.e. 3200mg/lit and 1600mg/lit of protein. Addition of glucose supplement increased the biomass production and 0.5% sugar gave maximum yield i.e. 5700mg/lit and 2800mg/lit of protein whereas further addition of glucose decreased the biomass production. Similarly, addition of pectin also positively influenced the growth of *Saccharomyces cerevisiae* at 0.5% concentration where maximum biomass was obtained i.e. 9800mg/lit or protein.

Key Words: Citrus peels, single cell protein, biomass production, agricultural waste

INTRODUCTION:

The continued population growth in developing countries has required an increase in animal and human food supply. The increasing world demand for protein rich food led to the search for the formulation of alternative protein sources to supplement the conventional protein sources. Single Cell Protein (SCP) is one of the most important steps for this goal and is an alternative and an innovative way to successfully solve the global food problem (Najafpur, Ghasem D., 2007).

The term SCP refers to dead, dry microbial cells or total proteins extracted from pure microbial cell culture and is produced using a number of different microorganisms including bacterium, fungus and algae (Anupama & Ravindra, 2000). It can also be called biomass, bioprotein or microbial protein. The word SCP is considered to be appropriate since most of the microorganisms grow as single or filamentous individuals. Besides high protein content (about 60-82% of dry cell weight), SCP also contains fats, carbohydrates, nucleic acids, vitamins and minerals (Asad M. *et al.*, 2000, Jamel P. *et al*, 2008). Another advantage with SCP is that it is rich in certain essential amino acids like lysine, methionine which are limiting in most plant and animal foods. This protein can be used as additive added to the main diet instead of sources known very expensive such as soybean and fish (Najafpur, Ghasem D.,2007: Gad, A. S. *et al.*, 2010).

India is the second major producer of fruits and vegetables in the world. It contributes 10% of world fruit production. According to India Agricultural Research Data Book 2004, the total waste generated from fruit and vegetables comes to 50 million tons per annum. Orange waste is one of the appealing nutrient sources for growth of microorganisms (Lopez *et al*., 2010: Kantifedaki *et al*., 2018). Fruit wastes rich in carbohydrate content and other basic nutrients could support microbial growth thus fruit processing wastes are useful substrate for production of microbial proteins. The utilization of fruit wastes in the production of SCP will help in controlling pollution and also solving waste disposable problem to some extent in addition to satisfy the world shortage of protein rich food (Anamika Malav *et al.*, 2017).

Yeasts are probably the most widely accepted and used microorganism for single cell protein. So, it will be beneficial to focus on yeast single cell protein rather than bacterial and algal single cell protein. Yeasts have a balance proportion of amino acids, B-complex vitamins and also having probiotic properties (Anamika Amata, I.A., 2013). Over the last few years, a lot of research has been done for reprocessing and reuse of different fruit wastes for the conversion of valuable and nutritive products (Singh *et al*., 2009: Mahmood Khan Yousuf., 2012: Ofodile L. *et al*., 2011). The industrial wastes of citrus processing plants contain peels, rag and seeds which are rich in carbohydrates, poor in proteins and account for about 45-60% the weight of fruits. The use of such cheap and readily available substrates is desirable to lower the cost of production, reduce waste disposal and management problems. Therefore, the present investigation was carried out to assess the potential of orange peels for cost effective biomass production using pectinolytic microorganisms.

MATERIALS AND METHODS:

MATERIALS:

Collection of orange peels: Orange peels were collected from fruit juice centers and local market of Parbhani city.

Microorganisms:

Three different groups of microorganisms were used in the first step viz. *Pseudomonas fluorescence* a bacterium, *Aspergillus oryzae* a fungus and the yeast *Saccharomyces cerevisiae*. All these cultures were procured from NCIM, NCL Pune. Bacterial culture was maintained on nutrient agar medium, fungus on potato dextrose agar slant and yeast on Sabouraud's dextrose agar slant. All cultures were sub cultured for every 15 days and stored at 4^0C.

Production Media:

Hankin's Mineral Medium described by Deshmukh, A.M. (2007) was used and instead of pectin, the powdered citrus waste (orange peel powder) was added as source of carbon.

METHODOLOGY:

Treatment of orange peels:

Orange peels were washed properly and then oven dried at 60^0C overnight and ground into fine powder by using electric grinder. The powder was used as substrate for further study.

Setting up of fermentation:

For Pseudomonas fluorescence;

5% and 10% of orange peel powder was weighed in separate sets and boiled into distilled water to get homogenous suspension. Filtered to remove the particulate traces of orange peels. The volume was made up to the mark required and remaining ingredients were added according to the specified medium. pH was adjusted according to requirement and sterilized at 121^0C for 15 minutes.

The *Pseudomonas fluorescence* culture was scrapped from the slant using saline and later 2% of this suspension was used as inoculum. Incubated at 37^0C for 4-5 days. After 5 days maximum amount of growth was obtained which was later subjected to further processing.

For Aspergillus oryzae:

Media was prepared as mentioned above. *Aspergillus oryzae* slants on PDA were observed for maximum sporulation. The culture was scrapped into saline until thick suspension of spores was prepared. This suspension was used as inoculum.

2% of spore suspension was inoculated into the sterilized medium. The flasks were incubated for 4-5 days, stationary and submerged. After incubation period maximum amount of growth was obtained, later used for further processing.

For Saccharomyces cerevisiae:
Media was prepared as mentioned above. *S. cerevisiae* maintained on Sabouraud's dextrose agar slants was scrapped into 10 ml saline and thick suspension was prepared.
2% of this suspension was inoculated into the medium and incubated for 5 days on rotary shakers. After 5 days, the biomass was collected from flasks.

Harvesting of biomass:
After incubation the biomass of *P. fluorescence* was collected by centrifugation at 7000 rpm for 20 minutes. The biomass was washed, filtered through Whatman filter paper and dried in oven until becomes dry.
A. oryzae contents of the flask were filtered through Whatman filter paper. The biomass was oven dried.
Yeast inoculated flasks were subjected to centrifugation at 7000 rpm for 20 minutes. Cell mass was washed, filtered and oven dried at 105^0C.

Extraction:
The dried biomass was ground in mortar and pestle along with Whatman filter paper and sterile fine sand in order to crush the cells properly. The ground material was centrifuged at 7000 rpm for 30 min. The supernatant was used for the estimation of protein.

Protein estimation:
Protein estimation of the extracted samples was done by Folin Lowry method using BSA as standard. (Lowry *et al*., 1951)
Effect of glucose and pectin supplement on biomass production by *S. cerevisiae* was also studied by the above-mentioned method.

RESULTS AND DISCUSSION:

As per (Fig. I), amongst the three tested organisms, *P. fluorescence* gave least biomass i.e. 1000 mg/lit whereas *S. cerevisiae* gave maximum biomass i.e. 3200 mg/lit using orange peels as substrate under submerged condition indicating that *S. cerevisiae* has highest ability to utilize orange peels as compared to *Pseudomonas* and *Aspergillus.*

Addition of glucose supplement increased the biomass production up to 0.5% w/v whereas further increase in glucose concentration decreased the biomass production. 0.5% glucose supplement in hydrolyzed media gave maximum biomass yield i.e. 5700mg/lit and 2088 ml/lit of protein. (Fig. II)

Similarly, additional pectin also positively influenced the growth of Saccharomyces cerevisiae at 0.5% concentration where maximum biomass was obtained i.e. 9800mg/lit (Fig. III). Fruit hydrolyzed media supplemented with 0.5% pectin (usual concentration of Hankin's media) showed more than 25fold increase in the biomass production by *S. cerevisiae*.

Khan, Dahot,2010 & Yalemtesfa *et al*, 2010; showed production of SCP on orange peel was 7500 mg /l and 440 mg/Lit whereas in the present investigation it was enhanced up to 9800mg/lit.

The addition of nutrient supplements (glucose) on the other hand greatly increased the SCP production of *S. cerevisiae*; which is similar to the studies reported by Mondal *et al.* (2012) and Adoki (2008).

CONCLUSION:

Results of this study indicated that citrus or orange peels can be effectively utilized as a substrate for production of single cell protein by *S. cerevisiae.*

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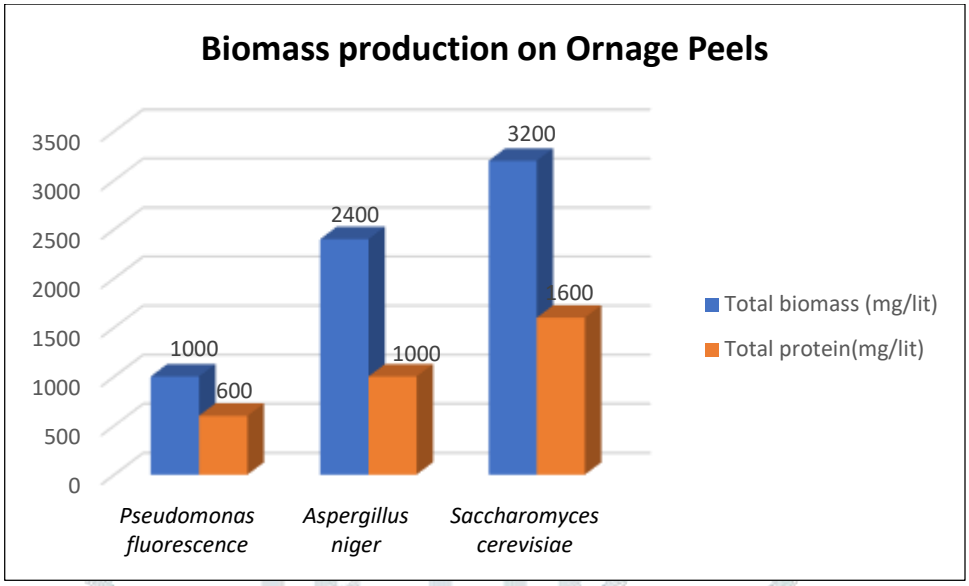
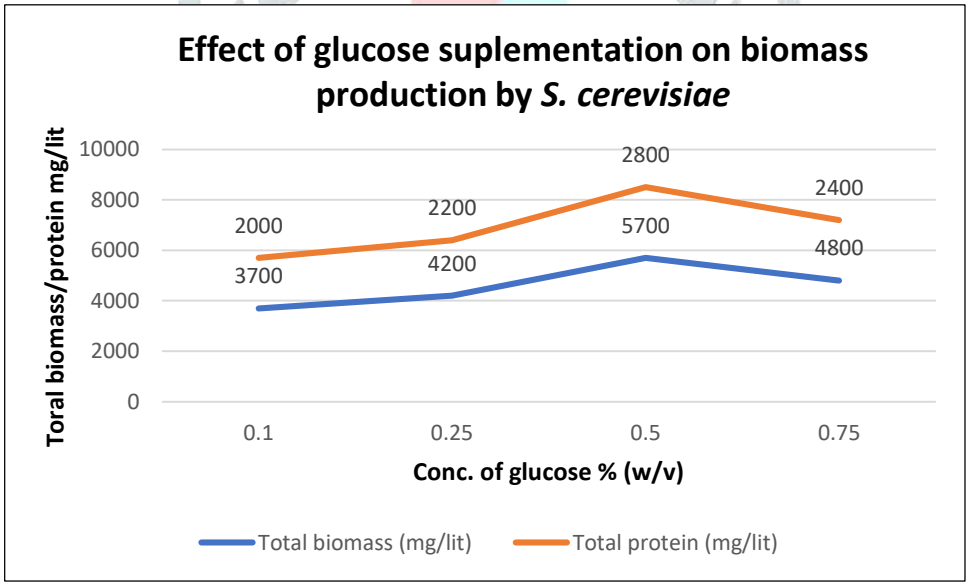
FIGURES:

I. Total Biomass production and protein content of Biomass under submerged condition.



II. Effect of Glucose supplementation on Biomass production by *S. cerevisiae*



III. Effect of pure pectin supplementation on biomass production by *S. cerevisiae*