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RESEARCH ARTICLE

PRODUCTION OF SINGLE CELL PROTEIN FROM PINEAPPLE WASTE USING YEAST

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Abstract

The worldwide food protein deficiency is becoming alarming day to day and with the fast growing population of world, pressure is exerted on the feed industry to produce enough animal feed to meet the region's nutritional requirements. 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, pineapple waste was used as sole carbon source in five concentrations for preparation of fermentation media on which two strains of yeasts, *Saccharomyces cerevisiae* and *Candida tropicalis* were grown. The increased concentration of pineapple hydrolysate enhanced the biomass yield and the protein formation within the yeast cells. Lower carbon utilization by the two yeast strains occurred in the waste-containing media, as compared to control, increasing the economic value of the waste obtained after 7-day fermentation. The present finding helps in SCP production from cheap, inexpensive agro waste material.

Key words: Yeast, SCP, Pineapple waste. *Saccharomyces cerevisiae, Candida tropicalis*

Introduction

A growing concern for the acute food shortages for the world's expanding population has led to the exploitation of non-conventional food sources as potential alternatives. Among these, the single cell organisms probably present the best chances for the development of unique independence of agricultural crop based food supply. The protein obtained from the micro-organisms is cheap and competes well with other sources of protein and may provide good nutritive value depending, however, upon the amino acid composition. But correction in malnutrition through high quality protein results in a higher cost of feeding. Interest in microbial production is increased because microorganisms can utilize waste material that causes pollution problems and or sanitary hazards. Agricultural waste is a renewable resource of great variety of biotechnological potential. In recent years waste likes bagasse, rice straw, rice hulls and starch residues has been used as substrates for growing microbes. If these bulk materials is economically useful to control the pollution problems and eliminate the waste disposal

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problem. The single cell protein is a dehydrated cell consisting of mixture of proteins, lipids, carbohydrates, nucleic acids, inorganic compounds and a variety of other non protein nitrogenous compounds such as vitamins. Agricultural wastes are useful substrate for production of microbial protein, but must meet the following criteria; it should be non toxic, abundant, totally regenerable, non-exotic, cheap and able to support rapid growth and multiplication of the organisms resulting in high quality biomass.

With the current population explosion and the limited land resources, the world will be soon unable to feed its population. At the present time the food problem is limited mainly to developing countries of Asia, Africa and South America. However, Predictions show that more advanced nations will eventually face the same problems. The developing of novel food production process independent of agricultural land use is thus becoming imperative.

The UN recently reported that over 1 billion people currently do not have access to adequate amounts of food, and that number could climb even higher in the near future if current drought conditions persist. Based on UN research, it was estimated a decrease in agricultural production of 20-40% if drought conditions continue and concluded that 2009 could be the beginning of a record-breaking humanitarian crisis throughout much of the world. The demand for protein as food is expanded for malnourished human populations. Consequently, the cost of protein secondary use as food for livestock has also risen. Increasingly there was always a stimulus needed to introduce an additional and or complementary source of animal feed microorganisms that are considered for food or feed use including algae, bacteria, yeasts, molds, and higher fungi. The dried cells of these organisms are collectively referred to as single cell protein. Different types of microorganisms have been recommended for human consumption, including yeast, molds and algae. But as of now only yeast have been used as food to any extent and then under unusual conditions. During Second World War when there were shortages in proteins and vitamins in the diet, the Germans produced yeast and mold *Geotrichum candidum* in some quantity for food.

Research on single cell protein has been stimulated by a concern over the eventual food crisis or food shortages that will occur if the world's populations are not controlled. Many scientists believe that use of microbial fermentations and the development of an industry to produce and supply single cell proteins from agricultural waste are insufficient.

The present study was focused on yeast single cell protein rather than bacterial, fungal and algal single cell protein. Algal single cell protein have limitations such as the need for warm temperatures and plenty of sun light in addition to carbon dioxide, and also that the algal cell wall is indigestible. Bacteria are capable of growth on a wide variety of substrates, have a short generation time and are high protein content. Their use is somewhat limited by poor public acceptance of bacteria as food, small size and difficulty of harvesting and high content of nucleic acid on a dried weight basis. Yeasts are probably the most widely accepted and used microorganisms for single cell protein. These include strains of *Candida utilis, C. arborea, C. pulcherrima* and *Saccharomyces cerevisiae.*

Pineapple is an important fruit crop, belonging to the family Bromeliaceae. In India nearly 100 different cultivars are known, of which 'kew' and 'Queen' are most useful and cultivated widely. In India estimates of the area under pineapple vary from 24,350 ha to 56,000 ha. The world shortage of protein has stimulated the interest of scientists for the production of unconventional protein – rich food and feed-stuffs. The production of single cell protein (SCP) by fermentation processes has been mentioned by many investigators and the impressive advantages of microorganisms for single cell protein (SCP) production compared with conventional sources of protein (soybeans or meat) are well known (Argyro *et al.*, 2006). A number of agricultural and agro industrial waste products have been used for the production of SCP and other metabolites, including orange waste, mango waste, cotton salks, kinnow-mandarin waste, barley straw, corn cops, rice straw, corn straw, onion juice and sugar cane bagasse (Nigam *et al.*, 2000), cassava starch (Tipparat *et al.*, 1995), wheat straw (Abou Hamed, 1993), banana waste (Saquido *et al.*, 1981), capsicum powder (Zhao *et* *al.*, 2010) and coconut water (Smith and Bull, 1976). The usage of such wastes as a sole carbon and nitrogen source for the production of SCP by microorganisms could be simply attributed to their presence in nature on large scale and their cheap cost.

In this work we intended to investigate the possibility of bioconversion of pineapple in to single cell protein (SCP) by using two yeast strains; Saccharomyces *cerevisiae* and C*andida tropicalis* grown on based media containing different concentration of pineapple hydrolysate.

Materials and Methods

Collection of Pineapple waste and yeast culture

The ripe, yellowish and firm pineapple fruits were obtained from Vellore market, Tamilnadu, India. The yeast culture such as *Saccharomyces cerevisiae* MTCC 463 and *Candida tropicalis* MTCC184 were obtained from Microbial Type Culture Collections (MTCC), Chandighar, India. The cultures were maintained on slant of yeast peptone dextrose medium(Yeast extract 10g, Dextrose 20g, Peptone 20g, Agar 20g, pH = 6.0, Distilled water 1000ml) and stored at 4^{o}C.

Biochemical analysis of pineapple waste

The pineapple fruits skin waste was weighed and peeled. The moisture, protein, non reducing and reducing sugar were determined by AOAC methods (AOAC, 1975). The total solids were determined as per the methods of Ranganna (1978) and Sadhu (2001).

Fermentation

Five media, other than control, were prepared consisting of the basal media (D-glucose-10.0g; $(NH_2)_2SO_4$ – 5.0g ; KH_2PO_4 – 1.0g ; $MgSo_4$, $7H_2O$ – 0.5g ; NaCl – 0.1g ; $CaCl_2$ – 0.1g ; Distilled water 1000 ml ; pH – 5.5 (Phaff *et al.*, 1996) free from glucoses but supplied with 1 to 5% pineapple hydrolysate. The medium were distributed in Erlenmeyer flasks and sterilized at 121^{o}C for 15 minutes. The Yeast strains were inoculated in the media and incubated at 28^{o}C for 7 days. The yeast cells were separated by washing from the fermented broth and analyzed.

Analysis of yeast biomass

The yeast was analyzed for its protein content using the procedure mentioned by Fawcett and Scott (1960). The reducing and non reducing sugar was analysed using the Nelson Somogyi and Anthrone methods, respectively (Krishnaveni *et al.*, 1984; Hedge *et al.*, 1962).

Results and discussions

The pineapple skin waste was found to contain a good amount of reducing and non-reducing sugars (10 and 13%, respectively), which is most favorable for the growth of microorganisms. It was further found to contain 0.6% protein. It was most suitable for yeast fermentation (Table 1).

Table 1. *Biochemical analysis of pineapple waste*

Characteristics	Percentage by weight
Moisture	85.0%
Reducing sugar	10.8%
Non reducing sugar	13.0%
Protein	0.6%

Bioconversion of pineapple waste

An attempt was made to study the bioconversion efficacy of pineapple waste into single cell protein using isolated yeast. For this study the basal medium was used with different concentration pineapple waste as a carbon source instead of glucose. After fermentations, the yeast cells were separated and washed with distilled water. The biochemical constituents of separated yeast cells were studied such as wet and dry biomass, protein contents and reducing sugar contents was evaluated.

Effect of pineapple waste hydrolysate on wet biomass of yeast Isolates

The effect of pineapple waste on wet biomass of yeast at 1% to 5% concentrations was studied. The results revealed that the wet biomass produced in pineapple medium was higher than basal medium. Among the two isolates, *S. cerevisiae* was recorded as highest wet biomass (4565 mg/100ml), followed by *C. tropicalis* (3480 mg/100ml). The highest wet biomass of *S. cerevisiae* was recorded on 7th day of fermentation at 5% concentration of pineapple waste.

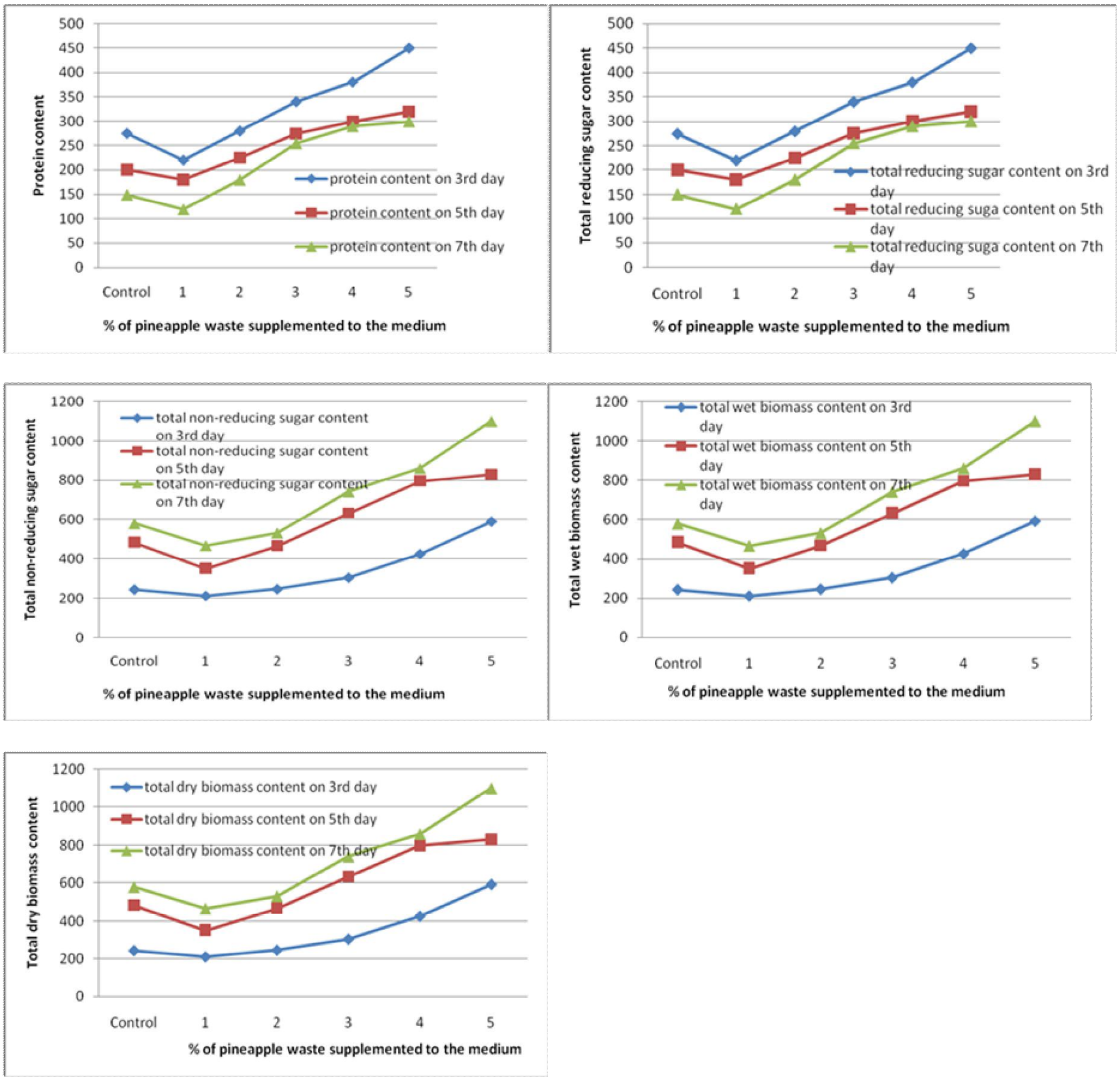

Figure 2. *Changes in media inoculated with S. cerevisiae throughout the fermentation period*

Effect of pineapple waste hydrolysate on dry biomass of yeast Isolates

The dry biomass content increased with increase in the concentration of carbon source. Among the two isolates, *S.cerevisiae* was recorded as highest dry biomass (571mg/100ml) followed by *C. tropicalis* (492mg/100ml). The highest dry biomass of *S. cerevisiae* was recorded on 7^{th} day of fermentation at 5% concentration of pineapple waste.

Effect of pineapple waste hydrolysate on protein content of yeast Isolates

The *S. cerevisiae* recorded high protein content (276mg/100ml). The protein content increased with increase in concentration of carbon source in the medium. The maximum protein content of *S.cerevisiae* was recorded on 3^{rd} day of the fermentation at 5% concentration of pineapple waste.

Effect of pineapple waste hydrolysate on reducing sugar of yeast Isolates

In general the reducing sugar content increased with the increase in the concentration of carbon source in the yeast basal medium. The content of reducing sugar in the yeast was reduced from 9.45 mg/100 ml to 3.36mg/100ml on 7^{th} day of observation on fermentation process. Among the two isolates used in the study *S. cerevisiae* recorded highest reducing sugar (7.91mg/100ml) followed by *C. tropicalis* (6.81mg/100ml). The maximum utilization of reducing sugar of *S. cerevisiae* was recorded on 3rd day fermentation at 5% waste concentration.

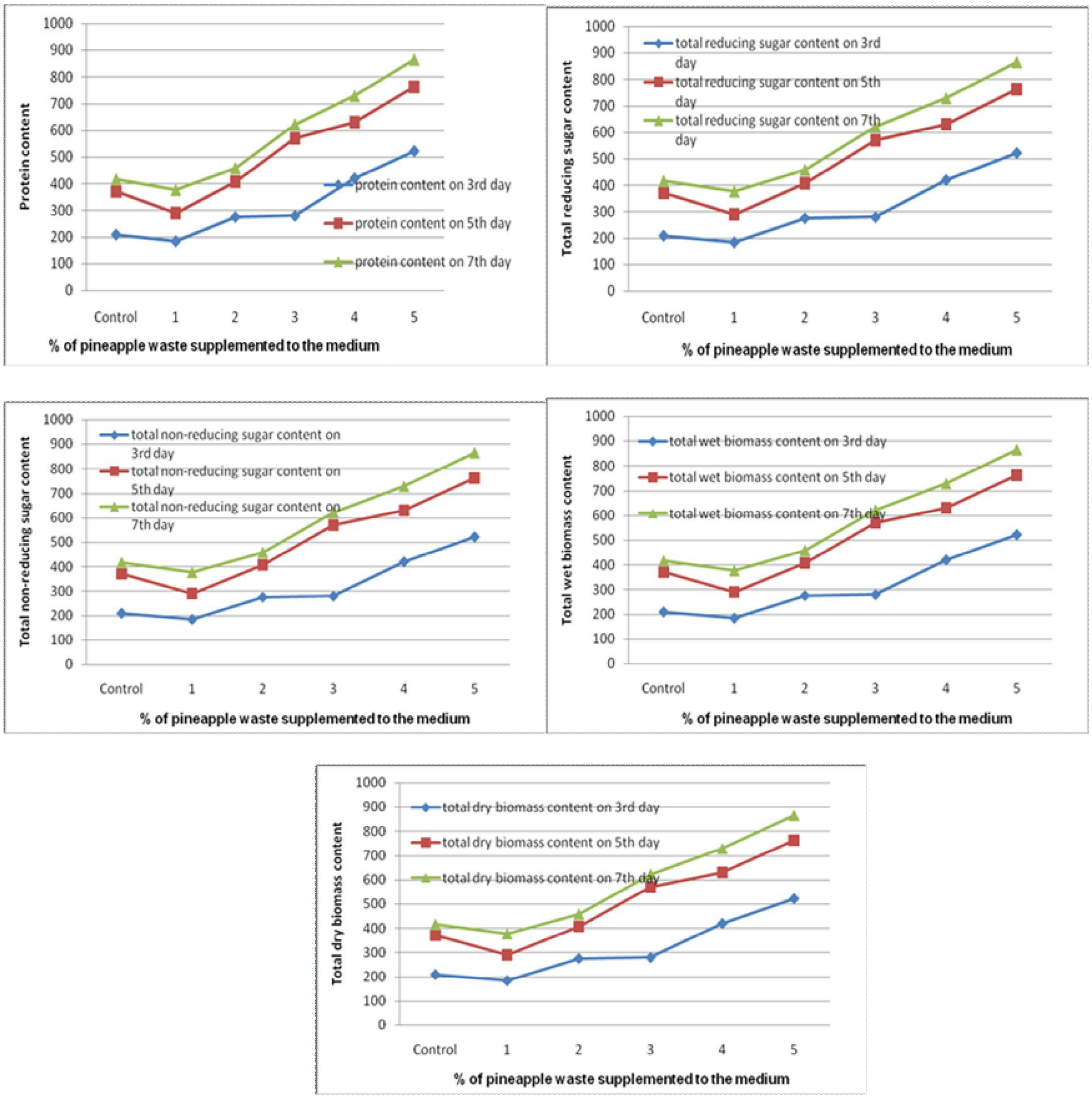

Figure 3. *Changes in media inoculated with C. tropicalis throughout the fermentation period*

Effect of pineapple waste hydrolysate on sugar utilization of non reducing sugar of yeast Isolates.

The result of sugar utilization increases with increase in concentration of carbon source with basal medium. The isolates *C. tropicalis* registered the highest total sugar utilization (319mg/100ml), followed by *S. cerevisiae* (308mg/100ml). The highest total sugar utilization (1237mg/100ml) was noted at the 7th day at 8% waste concentration when *S. cerevisiae* used as inoculum. (Figures 2 and 3).

Yeast is currently the most commonly used organism in the production of biomass, probably because it is already accepted both in human food and animal feed industries. Yeast based processes are the most advanced towards commercial production, followed by bacterial processes. Yeast may have many convenient characteristics, such as the ability to use a wide variety of substrates like hexose, pentose, and hydrocarbon, susceptibility to genetic variation, ability to flocculate and high nutritional value. In the present study an attempt was made to the study the bioconversion efficiency of pineapple waste into SCP by yeast.

Based on the fermentation observations the highest biomass (wet and dry biomass) was recorded on the 7th day of fermentation at 5% pineapple waste hydrolysate concentration, where *S. cerevisiae* was used as inocula. The same trend was found when *Saccharomyces sp* and *C. tropicalis* were used as inocula. This may be due to increased carbon source in the medium. Among the two organisms, *S.cerevisiae* is effective in utilization of carbon source from the pineapple waste when compared to *C. tropicalis*. These findings were in agreement with findings of Abou Hamed (1993).

The biomass level was increased with the increase in concentration of pineapple waste concentration, because the pineapple waste itself contains 13% sugar, 0.6% protein and trace level of calcium, phosphorous, ions and vitamins. Hence the availability of nutrients in pineapple has rapidly promoted growth of yeast cells. Concerning protein content, the highest protein content was recorded on the 3rd day of fermentation at 5% concentration and thereafter decreased. The present findings are in agreement with the findings of Abou Hamed (1993). The protein content increased as the concentration of wheat straw hydrolysate increased, at the same time the protein content gradually decreased when the fermentation period increased.

The highest reducing sugar content was recorded on 3rd day of fermentation at 5% hydrolysate waste concentration. It is obvious that sugar utilization was gradually increased as the fermentation time increased. The maximum sugar utilization occurred after 7th day fermentation. The maximum biomass yield was recorded after 7th day fermentation. The results corroborated the findings of Kalra *et al.*, (1989). The maximum yield of protein was attained in 5% waste concentration, at which concentration the yeast wet biomass contained about 450mg/100ml while yeast culture on control medium contained about 275mg/100ml in the case of *S. cerevisiae*. This agrees with the findings of Rashad *et al.*, (1990). The protein content was decreased on 5th and 7th day of fermentation. This may be due to depletion of nutrients and release of waste products from yeast. This finding agrees with the observations by Posser and Tough (1991).

Microbial upgrading of solid wastes is becoming increasingly attractive in view of stricter environmental regulations and unacceptability of alternative treatment methods. Although it is still the early to come up with any detailed feasibility studies, it is evident that local markets for protein feed supplements do exist to replace the currently imported soybean and fish meals. The exploitation of our agricultural wastes for microbial protein production will greatly eliminate, if not minimize the immense cost of wastes pollution control.

Conclusions

The bioconversion effect of pineapple waste into SCP was evaluated using yeast. The increase in biomass contents were observed when there was increase in pineapple waste concentration. The highest biomass content of *S. cerevisiae* and *C. tropicalis* was recorded on 7th day fermentation. The highest protein content of *S.cerevisiae* and *C tropicalis* was recorded on the 3rd day of fermentation at 5 % concentration. The highest reducing sugar content of yeast was recorded on 3rd day of fermentation at 5% concentration. The utilization of reducing sugar was increased with the increase in the concentration of substrates. The present findings reveals that pineapple waste can be used as effective alternate carbon source for SCP production.

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