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PRODUCTION OF SINGLE CELL PROTEIN FROM SUGARCANE USING FUNGI

P. V. Kamala Kumari*, Y. Srinivasa Rao, V. Rama Vaishnavi and D. Sowjanya

Vignan Institute of Pharmaceutical Technology, Duvvada, Visakhapatnam - 530049, Andhra Pradesh, India.

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Correspondence to Author:
Dr. P. V. Kamala Kumari

Associate Professor,
Vignan Institute of Pharmaceutical Technology, Duvvada, Visakhapatnam - 530049, Andhra Pradesh, India.

Email: kamalaparavastu@gmail.com

ABSTRACT: Single cell protein is a protein extracted from cultured algae, yeasts, or bacteria and used as a substitute for protein-rich foods, especially in animal feeds or as dietary supplements. Many types of animal feeds contain single cell proteins. It can also be called biomass, bioprotein or microbial protein. The word single cell protein which was coined in the 1960 is considered to be appropriate since most of the microorganisms grow as single or filamentous individuals. Besides its high protein content (about 60 - 82% of dry cell weight), single cell protein also contains fats, carbohydrates, nucleic acids, Vitamins and minerals. According to various investigations on the fermentations of fruit juices, it has been reported that they could be used as feed-stocks for the production of single cell protein (SCP) based on their level of sugar and ability to support the growth of yeasts. Single cell proteins thus, are the dried cells of microorganisms such as yeast that could be grown in large-scale culture systems for use as protein for human or animal consumption. The aim of this study was to investigate the possibility of bioconversion of sugarcane extract into single cell protein by using fungal strain *Aspergillus niger*.

INTRODUCTION: Single-cell proteins are the dried cells of microorganism, which are used as protein supplement in human foods or animal feeds. Microorganisms like algae, fungi, yeast and bacteria, utilize inexpensive feedstock and wastes as sources of carbon and energy for growth to produce biomass, protein concentrate or amino acids. Microorganisms and substrates used for the production of single cell protein as shown in **Table 1**. Since protein accounts for the quantitatively important part of the microbial cells, these microorganisms, also called single cell protein as natural protein concentrate [1].

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With increase in population and worldwide protein shortage the use of microbial biomass as food and feed is more highlighted. The increasing world deficiency of protein is becoming a main problem of humankind. Since the early fifties, intense efforts have been made to explore new, alternate and unconventional protein [2]. For this reason, in 1996, new sources mainly yeast, fungi, bacteria and algae named Single Cell Protein (SCP) as coined to describe the protein production from biomass, originating from different microbial sources.

Microbial biomass has been considered an alternative to conventional sources of food or feed. The worldwide, large-scale development of SCP processes has contributed greatly to the advancement of present day biotechnology. Research and development of SCP processes has involved work in the fields of microbiology, biochemistry, genetics, chemical and process engineering, food technology, agriculture, animal

nutrition, ecology, toxicology, medicine and veterinary science and economics [3]. In developing SCP processes new technical solutions for other related technologies in waste water treatment, production of alcohol, enzyme technology and nutritional science also improves. The future of SCP will be heavily dependent on reducing production costs and improving quality by fermentation, downstream processing and improvement in the producer organisms as a result of conventional applied genetics together with recombinant DNA technologies [4]. Single cell proteins have application in animal nutrition as: fattening calves, poultry, pigs and fish breading in the foodstuffs area as: aroma carriers, Vitamin carrier, emulsifying aids and to improve the nutritive value of baked products, in soups, in ready-to-serve meals, in diet recipes and in the technical field as: paper processing, leather processing and as foam stabilizers.

TABLE 1: MICROORGANISM AND SUBSTRATES USED FOR SINGLE CELL PROTEIN PRODUCTION

Microorganism	Substrate
Bacteria	
Aeromonas hydrophylla	Lactose
Acromobacter delvacvate	n-alkanes
Acinetobacter calcoacenticus	Ethanol
Bacillus megaterium	Non protein nitrogenous compound
Bacillus subtilis, Cellulomonas sp.,	Cellulose, Hemicellulose
Flavobacterium sp., *Thermomonospora fusca*	
Lactobacillus sp.	Glucose, Amylose, Maltose
Methylomonas methylotrophus, M. clara	Methanol
Pseudomonas fluorescens	Uric acid and other non – protein Nitrogenous compound
Rhodopseudomonas capsulata	Glucose
Fungi	
Aspergillus fumigatus	Maltose, Glucose
Aspergillus niger, A. oryzae,	Cellulose, Hemicellulose
Cephalosporium eichhorniae, Chaetomium cellulolyticum	
Penecillium cyclopium	Glucose, Lactose, Galactose
Rhizopus chinensis	Glucose, Maltose
Scytalidium aciduphlium, Thricoderma viridae, Thricoderma alba	Cellulose, Pentose
Yeast	
Amoco torula	Ethanol
Candida tropicalis	Maltose, Glucose
Candida utilis	Glucose
Candida novellas	n- alkane
Candida intermedia	Lactose
Sachharomyces cereviciae	Lactose, Pentose, Maltose
Algae	
Chlorella pyrenoidosa, Chlorella sorokiana, Chondrous crispus,	Carbon dioxide through
Scenedesmus sp., *Spirulina* sp., *Porphyrium* sp.	photosynthesus

MATERIALS AND METHODS:

Collection and Preparation of Substrates: Sufficient amount of sugarcanes were bought from the local markets of Visakhapatnam, Andhra Pradesh, India. The sugarcane peels were separated and thoroughly washed with sterile water and were placed in a blender to form a pure extract of sugarcane, this extract was passed through a mesh screen. The sample thus prepared was zip-locked in transparent polythene bags and stored at lower temperatures in a freezer until further study.

Procedure for Single Cell Protein (SCP) production: The obtained extract was taken and transferred to pre-sterilized conical flask, was then autoclaved at standard temperature of 121 °C, pressure of 15 psi for a time period of 15 minutes. After autoclaving, the sterilized sugar cane extract was aseptically transferred into pre-sterilized Petri-plates [5]. Then upon cooling, in aseptic conditions the Petri plates containing sugar cane extract were inoculated with standard filamentous fungi *i.e.*, *Aspergillus niger.* These Petri plates were then incubated at 28 °C for 7 days. After the growth of fungal biomass, the mycelia were transferred on a filter paper (Whatman filter paper) and washed with distilled water if any. The filter papers

containing the mycelia were dried at 90 °C for 24 hrs to get moisture free fungal content [6].

Microorganism: The microorganisms used to ferment sugar cane extract was *Aspergillus niger* obtained from Department of Biotechnology, Vignan Institute of Pharmaceutical Technology, Visakhapatnam. The fungi was grown on Sabouraud's dextrose agar media and incubated at a temperature of 28 - 30 °C for a period of 2 weeks.

Chemical Analysis: The reducing sugars were determined using DNS reagent and the total carbohydrate content was determined using Anthrone reagent. Moisture content was determined by the method based on principle of drying to constant weight. The biomass was expressed in terms of protein content. The protein estimation was determined according to Modified Biuret's method.

RESULTS AND DISCUSSION: The sugarcane extract was found to contain a good amount of reducing and non-reducing sugars (10.5 and 12.2%, respectively), which is most favourable for the growth of microorganisms. It was further found to contain 0.8% protein as shown in **Table 2**.

TABLE 2: BIOCHEMICAL ANALYSIS OF SUGARCANE EXTRACT

Characteristics	Percentage by weight
Moisture	80%
Reducing sugar	10.5%
Non reducing sugar	12.2%
Protein	0.8%

Bioconversion of Sugarcane Extract: An attempt was made to study the bioconversion efficacy of sugarcane extract into single cell protein using isolated fungi. For this study the basal medium was used with different concentration sugarcane extract as a carbon source instead of glucose [7]. After fermentations, the fungal cells were separated and washed with distilled water. The biochemical constituents of separated fungal cells were studied such as wet and dry biomass, protein contents and reducing sugar content [8].

Effect of Sugarcane Extract on Wet Biomass of Fungal Isolate: The effect of sugarcane extract on wet biomass of fungi at 1% to 5% concentrations was studied. The results revealed that the wet biomass produced in sugarcane medium was higher than basal medium. Wet biomass of 1000 mg/100ml was obtained and it was recorded on 7th day of fermentation at 5% concentration of sugarcane extract shown in **Table 3** and **Fig. 1**.

TABLE 3: EFFECT OF SUGARCANE ON WET BIOMASS (mg/100ml) OF FUNGAL ISOLATE

Effect of sugarcane on wet biomass (mg/100ml) of fungal isolate			
% of sugarcane supplied to basal media	Incubation period (Days)		
	3rd Day	5th Day	7th Day
Control	200	450	580
1	150	358	480
2	170	460	560
3	250	580	750
4	352	690	800
5	520	698	1000

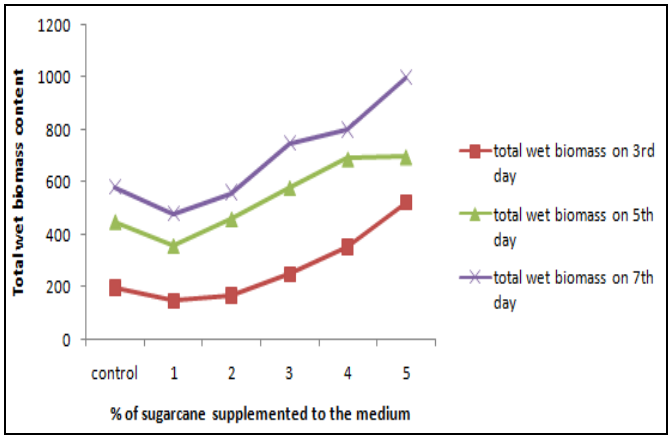

FIG. 1: EFFECT OF SUGARCANE ON WET BIOMASS (mg/100ml) OF FUNGAL ISOLATE

TABLE 4: EFFECT OF SUGARCANE ON DRY BIOMASS (mg/100ml) OF FUNGAL ISOLATE

Effect of sugarcane on dry biomass (mg/100ml) of fungal isolate			
% of sugarcane supplied to basal media	Incubation period (Days)		
	3rd Day	5th Day	7th Day
Control	210	500	590
1	190	340	450
2	225	460	540
3	300	540	700
4	352	600	790
5	460	700	1100

Effect of Sugarcane Extract on Dry Biomass of Fungal Isolate: The dry biomass content increased with increase in the concentration of carbon source. The results revealed that the dry biomass produced in sugarcane medium was higher than basal medium. Dry biomass of 1100 mg/100ml was obtained and it was recorded on 7th day of

fermentation at 5% concentration of sugarcane extract shown in **Table 4** and **Fig. 2**.

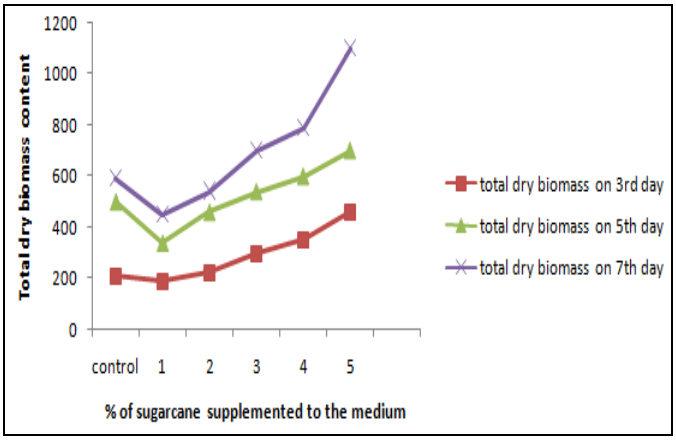

FIG. 2: EFFECT OF SUGARCANE ON WET BIOMASS (mg/100ml) OF FUNGAL ISOLATE

Effect of Sugarcane Extract on Protein Content of Fungal Isolate: Protein content of 450mg/100ml was recorded on 3rd day of the fermentation at 5% concentration of sugarcane extract. The protein content increased with increase in concentration of carbon source in the medium shown in **Table 5** and **Fig. 3**.

TABLE 5: EFFECT OF SUGARCANE ON PROTEIN CONTENT (mg/100ml) OF FUNGAL ISOLATE

Effect of sugarcane extract on protein content (mg/100ml) of fungal isolate			
% of sugarcane supplied to basal media	Incubation period (Days)		
	3rd Day	5th Day	7th Day
Control	275	200	150
1	225	175	100
2	275	235	170
3	350	250	250
4	375	285	275
5	450	300	286

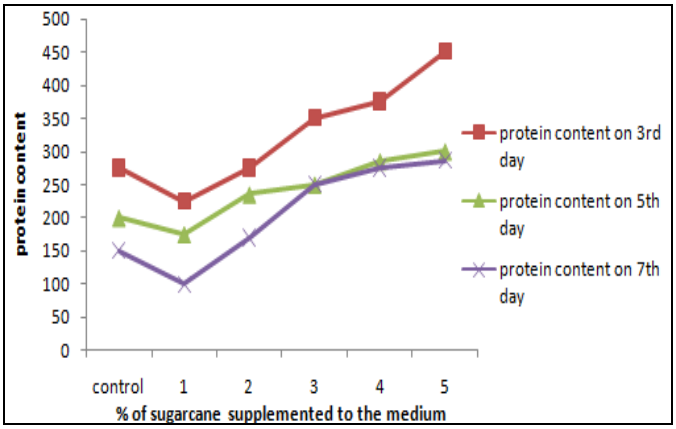

FIG. 3: EFFECT OF SUGARCANE ON PROTEIN CONTENT (mg/100ml) OF FUNGAL ISOLATE

Effect of Sugarcane Extract on Reducing Sugar of Fungi Isolate: In general the reducing sugar content increased with the increase in the concentration of carbon source in the basal medium [9]. The maximum utilization of reducing sugar of fungi (450/100ml) was recorded on 3rd day fermentation at 5% concentration as shown in **Table 6** and **Fig. 4**.

TABLE 5: EFFECT OF SUGARCANE ON REDUCING SUGAR CONTENT (mg/100ml) OF FUNGAL ISOLATE

Effect of sugarcane extract on reducing sugar content (mg/100ml) of fungal isolate			
% of sugarcane supplied to basal media	Incubation period (Days)		
	3rd Day	5th Day	7th Day
Control	250	200	150
1	200	175	140
2	285	235	160
3	300	250	225
4	350	280	250
5	450	300	275

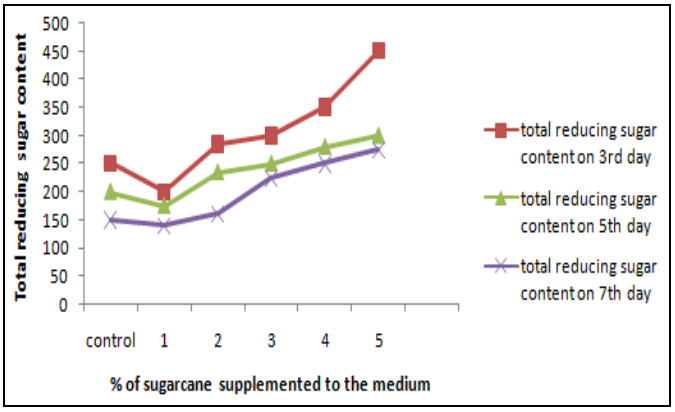

FIG. 4: EFFECT OF SUGARCANE ON REDUCING SUGAR CONTENT (mg/100ml) OF FUNGAL ISOLATE

Effect of Sugarcane Extract on Non-Reducing Sugar of Fungal Isolate: The result of sugar utilization increases with increase in concentration of carbon source with basal medium. The highest total sugar utilization (1200mg/100ml) was noted at the 7th day at 5% concentration shown in **Table 7** and **Fig. 5**.

Based on the fermentation observations the highest biomass (wet biomass 1000mg/100ml and dry biomass 1100mg/100ml) was recorded on the 7th day of fermentation at 5% sugarcane extract concentration, where *Aspergillus niger* was used as inoculum. Concerning protein content, the highest protein content of 450mg/100ml was recorded on the 3rd day of fermentation at 5% concentration and

thereafter decreased. The highest reducing sugar content (450mg/100ml) was recorded on 3rd day of fermentation at 5% concentration. It is obvious that sugar utilization was gradually increased as the fermentation time increased. The maximum sugar utilization (1200mg/100ml) occurred after 7th day fermentation.

TABLE 7: EFFECT OF SUGARCANE ON NON-REDUCING SUGAR CONTENT (mg/100ml) OF FUNGAL ISOLATE

Effect of sugarcane on non-reducing sugar content (mg/100ml) of fungal isolate			
% of sugarcane supplied to basal media	Incubation period (Days)		
	3rd Day	5th Day	7th Day
Control	210	400	550
1	200	350	475
2	225	375	490
3	250	475	750
4	300	500	800
5	350	710	1200

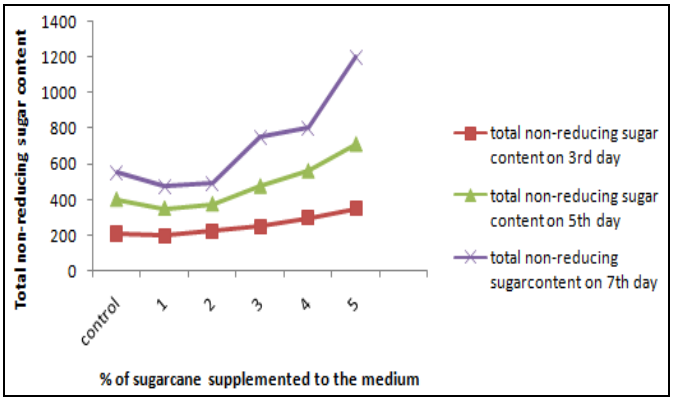

FIG. 5: EFFECT OF SUGARCANE ON NON- REDUCING SUGAR CONTENT (mg/100ml) OF FUNGAL ISOLATE

CONCLUSION: The bioconversion effect of sugarcane extract into SCP was evaluated using fungi. The biomass level was increased with the increase in concentration of sugarcane extract concentration, because the sugarcane waste itself contains 12.2% sugar, 0.8% protein [10]. Hence the availability of nutrients in sugarcane has rapidly promoted growth of fungal cells. The highest biomass (wet biomass 1000mg/100ml and dry biomass 1100mg/100ml) was recorded on the 7th day of fermentation at 5% sugarcane extract concentration, where *Aspergillus niger* was used as inoculum. The present findings reveal that sugarcane extract can be used as effective alternate carbon source for SCP production.

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CONFLICTS OF INTEREST: Nil

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