Optimization of SCP production of *Aspergillus niger* using different fruit peels

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ABSTRACT

An attempt was made to apply solid state fermentation for the production of single cell protein using fruit peels of Banana, orange and papaya peels respectively and their different combinations were used for optimization of single cell protein production of *Aspergillus nige* at different pH and incubation period. Substrate of fruit peel was used in different combination to maximize protein content in *A.niger*. 4th combination gave the best result of maximum production of 1.352 mg/ml of protein on 8 days of incubation and at pH 7 which is directly related to maximum fungal growth.

Keywords: Single cell protein (SCP), Aspergillus niger

BACKGROUND

SCP is a term coined to embrace microbial biomass products which were produced by fermentation. SCP production by technology arose as a promising way to solve the problem of the worldwide shortage of protein. They evolved as bioconversion process which turned waste into products which added nutritional and market value. Intensive research into fermentation science and technology for biomass production as well as feeding has resulted in a profound body of knowledge, the benefits of which now span far beyond the field of SCP production. These agricultural products out marketed SCP on the grounds of lower price. The combination of sophisticated products with food processing technology yielded a new generation of SCP products which may be used as meat substitutes, texture providing agents and flavor enhancers. Future application of heterologous protein expression may further develop the potential of this food line resulting in precisely tailored products which meet specific dietary requirements or stimulate high added value specialty products(Ugalde and Castrillo,2001).

In year (Anupama,2001) studied the bioconversion of agricultural and industrial waste to protein rich food and fodder stock. SCP production has the potential for feeding the ever increasing world population at cheaper rates(Najafpour,2007)

Suggested the potential of SCP to overcome shortage of food in the world. The present investigation was carried out to utilize various combinations of fruit wastes in the production of SCP by using standard food fungi *Aspergillus niger.*

MATERIALS AND METHODS

Collection of culture

Pure culture of *Aspergillus niger (MCCB-0201)* was collected from research laboratory, Department of Microbiology and Fermentation Technology, JSBB, SHIATS, Allahabad. The culture was maintained on PDA media. The slants were grown at 28±2°C for 7 days.

Pretreatment of substrate

Banana, orange and papaya peels were collected from the local market. It was pretreated to expose cellulose fibers to cellulolytic attack by the organism. The pretreatment process was carried out by adding 1% sodium hydroxide to 10g of fruit waste and autoclaving at 121°C and at 15 psi for 30 min. Pretreated material was allowed to cool, subsequently filtered and washed to neutral pH.. Then it was dried at 60°C in an oven for 12 h. Later on it was used for solid state fermentation(Anupama,2001).

Media and cultivation for solid state fermentation

The fermentation was carried out in 250ml flask containing 10g of solid substrate in various combinations of fruit waste. There were four different combinations used in different ratios (Table 1). The content of the flask were mixed thoroughly and autoclaved at 121°C for 20 min. Each experiment was done in triplicate.

Table 1: Various combinations of substrate used during the experiment

Combinations	Ratio of substrates	Orange	Banana	Papaya
1st combination	1:1:1	3.33g	3.33g	3.33g
2nd combination	1:1:2	2.5g	2.5g	5.0g
3rd combination	2:1:1	5.0g	2.5g	2.5g
4th combination	1:2:1	2.5g	5.0g	2.5g

Inoculation: After cooling, the production media was inoculated with the 10^6 spores/ml of inoculums and incubated under constant condition at 30°C for 4 days.

Buffer extraction: 50 ml of carbonate bicarbonate buffer (0.1 M at pH 10.6) was added to the flask and then culture was homogenized for 30 minute using rotator shaker at 250 rpm then the content was squeezed through the distilled water wet muslin cloth. The filtrate was centrifuged at 5000 rpm for 10 minutes then supernatant was filtered through a whatman no.1 filter paper(Anupama,2001)

Optimization: Various combinations of substrates were made of different ratios, the fermentation media was incubated for 2days, 4 days, 6 days, 8 days and 10 days and fermentation media was incubated also at different pH of 4, 6, 7 and 8. Since *Aspergillus niger* grows best within the pH range of 5-7 to see the effect of incubation time on the single cell protein.

Protein estimation: Total protein estimation was done according to Lowry method(Lowry,1951).

RESULTS AND DISCUSSION

The concentrations stated in the present investigation was very much similar to the values revealed by(Ojokoh and Uzeh,2005) for papaya peel(Yalemtesfa et al) for orange and(Yabaya and Ado ,2008) for banana peel.

Table 2: SCP (mg/ml) content without fermentation

Concentrations	Orange peel	Banana peel	Papaya peel
0.1 ml	0.363	0.349	0.358
0.2 ml	0.416	0.570	0.458

Effect of different combinations on single cell protein:

Various combinations of different ratios of substrates were taken to check its effect on overall protein production. There were four different combinations used during the experiment.

Table 3: SCP (mg/ml) content in combinations of fruit peels without fermentation

Concentrations	1st combination	2nd combination	3rd combination	4th combination
0.1 ml	0.124	0.118	0.146	0.159
0.2 ml	0.194	0.204	0.226	0.205

Out of all, 4th combination showed greater protein content thus, the results shown by these combinations can be of greater interest in producing SCP and will show potential results on its application as feed or food. The maximum SCP production of various combinations (Table 3) for 0.1ml and 0.2ml volume of sample were given as followed:

Table 4: SCP (mg/ml) content in combinations of fruit peels after fermentation

Concentration	1st combination	2nd combination	3rd combination	4th combination
0.1 ml	0.774	0.790	0.608	0.840
0.2 ml	1.340	1.130	1.126	1.352

(Table 4) It determined that maximum SCP production occurred in the 4th combination i.e. 1.352 mg/ml in 0.2 concentrations of sample aliquots. All the combinations

showed more SCP production than pure substrates during the investigation. This finding can be applied on large scale up and low cost technology alternatives should be explored intensively. Furthermore, the biomass produced from fruit waste can be evaluated for its suitability to be used either as a biofertilizer or as animal feed.

It should be noted that the 4th combination where banana peel greater ratio in comparison to orange and papaya peels (2:1) showed more favorable results than rest of the combinations(Yousufi,2012) showed more SCP production when banana peel was in combination with other substrates.

Effect of incubation period

Increase in protein production was initiated at early stages of incubation and reached a peak of maximum production at 8th day. It was observed that SCP declined with prolonged incubation which could be due to loss of nutrient and accumulation of toxic compound in the medium. It was found that protein of pure substrates as banana, papaya and orange showed maximum productions on 3 day i.e. 72 hours (Yalemtesfa *et al* ,2010).

Table 5: Effect of incubation period on SCP (mg/ml) production using fruit peels

Incubation time	Orange peel		Banana peel		Papaya peel	
	0.1 ml	0.2 ml	0.1 ml	0.2 ml	0.1 ml	0.2 ml
1st day	0.321	0.382	0.109	0.156	0.308	0.360
2th day	0.328	0.463	0.258	0.317	0.413	0.521
3rd day	0.620	0.984	0.613	0.766	0.630	0.812
4th day	0.384	0.528	0.358	0.378	0.457	0.584

The present investigation reveals similar results shown by some researcher's as in the case of orange peel (Yalemtesfa *et al* ,2010) and (Khan and Dahot,2010) showed production of SCP on orange peel was 0.75 g/l and 0.44 mg/ml whereas in the present investigation it was 0.62 mg/ml. Then in case of banana peel(Kandari and Gupta ,2012) showed the biomass production in banana peel was 0.365 mg/ml and current result was found to be 0.613 mg/ml of the sample. This investigation had more protein content because of the type of strain used. In papaya peel protein content was found to be 0.630 mg/ml, similarly,(Khan et al ,2010)revealed 0.595 mg/g of substrate. The change either increase or decrease in the concentration is due to the change in substrates and conditions during the experiment.

It should be noted that the protein content on the 1-2 days is not much thus, the fungi is in its lag phase and then there is a sudden growth of the organism leading to

growth in biomass on the 3rd day. And at the 4th day it shows the declining phase where the biomass produced decreases due to low concentration of substrates (Yalemtesfa et al ,2010) . It (Table 6) consists of SCP production on the mentioned combinations (as given in Table 3) for different incubation period i.e. 2, 4, 6, 8 and 10 days. The following results are obtained below:

Table 6: Effect of incubation period on SCP (mg/ml) production using different combinations of substrates

Incubation time	1st combination		2nd combination		3rd combination		4th combination	
	0.1ml	0.2ml	0.1ml	0.2ml	0.1ml	0.2ml	0.1ml	0.2ml
2th day	0.128	0.197	0.125	0.207	0.143	0.248	0.161	0.198
4th day	0.162	0.274	0.142	0.180	0.118	0.190	0.158	0.286
6th day	0.429	0.889	0.655	0.981	0.541	0.936	0.434	0.732
8th day	0.714	1.342	0.764	1.126	0.595	1.130	0.841	1.352
10th day	0.639	1.122	0.547	0.910	0.496	0.940	0.618	1.126

It determined that the exponential fungal growth was observed from the 6th day which showed maximum protein enrichment was found on 8th day and after that on 10th day the declining phase started due to low substrate concentration. The optimum time for maximum protein production was determined to be on 8th day.

Effect of pH

On fermentation of the substrates and its combinations at different pH of 4, 6, 7 and 8, it was found that at 7 pH protein production was at its maximum(Yalemesfa *et al* ,2010) also reported the maximum production at pH 7. However, the best growth for *Aspergillus niger* lies within the range of 5-7.

Table 7: Effect of pH on SCP (mg/ml) production using fruit peels

pH	Orange peel		Banana peel		Papaya peel	
	0.1 ml	0.2 ml	0.1 ml	0.2 ml	0.1 ml	0.2 ml
4	0.344	0.690	0.580	0.720	0.390	0.773
6	0.533	0.814	0.605	0.686	0.544	0.815
7	0.590	0.906	0.610	0.733	0.740	0.970
8	0.465	0.730	0.330	0.450	0.520	0.740

It should be noted that, on optimizing the pure peels (substrate) at different pH showed maximum production favored at pH 7 and then at pH 6 showing the second best growth. It determined that the strain *Aspergillus niger* grows best within the pH range of 5-7 (Yalemtesfa *et al* ,2010)

Table 8: Effect of pH on SCP (mg/ml) production using different combinations of substrates

pH	1st combination		2nd combination		3rd combination		4th combination	
	0.1 ml	0.2 ml	0.1 ml	0.2 ml	0.1 ml	0.2 ml	0.1 ml	0.2 ml
4	0.380	0.632	0.443	0.889	0.392	0.785	0.436	0.741
6	0.393	0.636	0.456	0.896	0.504	0.890	0.537	0.943
7	0.713	0.974	0.718	1.120	0.595	0.982	0.841	1.126
8	0.455	0.890	0.644	0.981	0.541	0.936	0.645	1.001

During the experiment pH 4, 6, and 8 was taken into consideration with reference to the neutral pH 7. Thus, maximum protein content was seen at pH 7 then at pH 8. Thus, it clearly showed that *Aspergillus niger* strain best grows at basic pH and mostly at neutral pH i.e. 7.

On fermentation of various combinations (Table 3) it again reveals that *Aspergillus niger* grows best within the pH range of 5-7(Yalemtesfa *et al* ,2010) showed maximum growth with the same strain at pH 7 and so in the present investigation which also clearly depict the same showing maximum growth at pH 7 and then at pH 8 and so on. Knowing that, supplementation of the substrate with nitrogen source can easily be done for potential result in producing SCP.

Comparison of SCP (mg/ml) production between best incubation time and pH:

The 4th combination gives the maximum production of SCP due to its cellulose and carbohydrate content which makes it acts as a good carbon source. Usually all the combinations produced more SCP in comparison between incubation time and pH, from the table below (Table 9) showed that combinations when used may prove to be favorable for the fungal biomass production or SCP production as a whole.

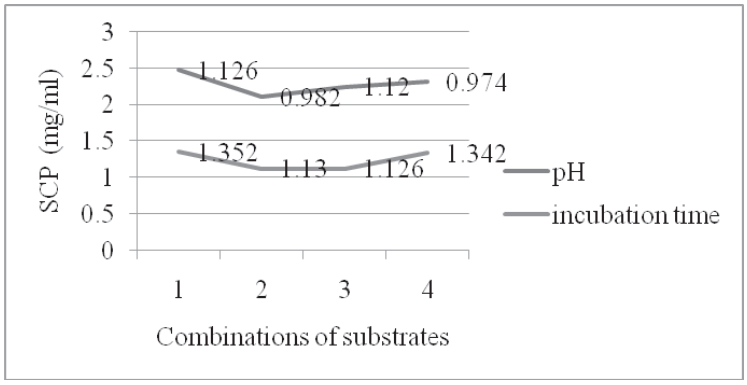

Figure 1: SCP (mg/ml) production of various combinations of substrates

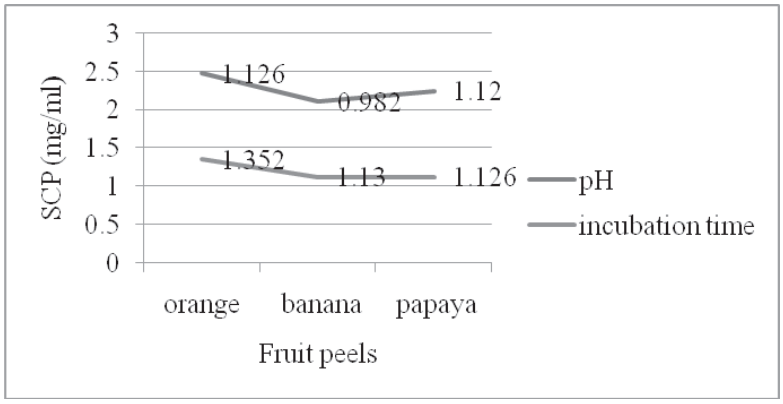

Figure 2: SCP (mg/ml) production of fruit peels

Table 9: Comparison of SCP (mg/ml) production between best incubation time and pH

Substrates used for fermentation	Protein concentration (mg/ml)at different incubation period	Protein concentration (mg/ml) at different pH
1st combination	1.342 (8th day)	0.974 (7)
2nd combination	1.126 (“)	1.120 (7)
3rd combination	1.130 (“)	0.982 (7)
4th combination	1.352 (“)	1.126 (7)
Orange peel	0.984 (3rd day)	0.906 (7)
Banana peel	0.766 (“)	0.733 (7)
Papaya peel	0.812 (“)	0.970 (7)

Aspergillus niger on 4th combination gave the best result of maximum production of 1.352 mg/ml of protein on 8 days of incubation and at pH 7and maximum fungal growth was observed which directly relates to protein content of *A.niger* .

Fruit peels which are considered as waste product that causes environment pollution. So fruit peels can be used in different combinations to produce SCP in enhanced nutritive value i.e. protein in different ratio as compared to individual fruit peels. Combination show enhanced protein content in biomass due to available substrates from different fruit peels. Fungal biomass utilizes different pathways to utilize different substrate to the fullest which is established by different combinations. The degree of mycelia biomass growth depends on the substrate utilized. Combinations provide greater protein content as compared to pure substrates. Out of the four combinations the best result was shown by the 4th combination which contained orange, banana and papaya peels in 1:2:1 ratio and the amount of total protein content in SCP of the 4th combination was 1.352mg/ml of the sample.

Thus present investigation might be of great help in making use of the fruit waste for sustainable management of fruit peels for economical and replenishment of food (nutrition scarcity) for poor and populous country like India.

CONCLUSION

From the present finding we conclude that the biomass production using different parameters (incubation time, and pH) and fruit peels combinations showed better results in comparison to fruit peels individually . Hence attempt for optimizing different combinations of fruit peels for SCP production seems to be successful and has a potential to be used for SCP for various applications and sustainable development.

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