

BIOSYNTHESIS OF SINGLE CELL PROTEIN BY YEAST STRAIN UNDER SUBMERGED CONDITION

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ABSTRACT

Agricultural residues rich in carbohydrates can be utilized in fermentation processes to produce microbial protein which in turn can be used to upgrade both human and animal feeds. The present study was planned to assess the feasibility of using fruit wastes for single cell protein production. For this purpose, twelve different single cell protein producing yeast strain isolated from different fruits and soil samples. The eight different substrates were studied for SCP production, among them banana peel gave the highest protein concentration. The potent yeast Candida Krusei (HSP₉) produced the highest protein content (4.52 g/l) at pH 5.5. Protein content (4.84g/l) was noted the highest when they were cultivated at a temperature of 37°C. The optimum culture density for SCP production was 6% V/V with the highest protein productivity (5.22g/l). An increase in substrate concentration the protein content of the biomass increased and reached the maximum (4.73g/l) at 6% (W/V) substrate concentration. The protein content also increased in ordered manner up to the maximum protein yield (5.0 g/l) at 72h incubation period. The maximum growth rate and protein productivity (8.76g/l) was realized at an agitation speed of 120 rpm at 37°C throughout the fermentation period of 72h. Medium containing (NH₄)₂SO₄ at 4.0g/l concentration was highly stimulated SCP production to yield the maximum protein concentration (11.64g/l). The maximum protein yield (24.30g/l) was obtained at 10g/l peptone concentration. The protein content in Candida Krusei (HSP₉) was increased when the glucose concentration was increased from 2.0 to 10g/l. It was found that at the end of complete optimization study, the maximum SCP production 11.54g/l was observed compared to unoptimized condition (4.5g/l).

KEY WORDS: Biosynthesis of SCP, Single cell protein (SCP), Submerged fermentation, Yeast Extract Glucose Chloramphenicol Agar plates (YGCA)

INTRODUCTION

Single cell protein (SCP) refers to sources of mixed protein extracted from pure or mixed culture of algae, yeast, fungi or bacteria (grow on different wastes) used as a substitute for protein rich food, in human and animal feeds. The term SCP was coined in 1966 by Prof. Carroll L. Wilson (Robert, 1989). Human being used microorganisms since prehistoric times.

Since 2500 BC, yeasts have been used in bread and beverage production. The alarming rate of population growth has increased the demand for food production. Increasing concern about pollution that occurs from agriculture and industrial wastes has stimulated interest in converting waste material into commercially valuable products. Single cell organisms probably present the best chance for the development

of unique independence of agricultural based food supply. Dhanasekeran *et al*, (2011). Thus, production of microbial proteins or bioprotein by fermentation of agricultural waste products is one of the most promising approaches for increasing the availability of proteins in the world. The raw material used as a substrate for SCP production is usually agriculture, forestry and food waste by-products. There are two types of raw materials depending on the grown microorganisms: conventional materials like starch, molasses, whey, fruit and vegetable wastes, wood, straw etc., and unconventional like petroleum byproducts, natural gas, ethanol, and methanol etc. (Jay, 1996). Agriculture waste such as banana peel, one of the main byproducts of banana cultivation and processing, which in most cases discarded into the environment where it caused environmental fouling. Banana peel contained almost all the essential nutrients needed for microbial growth; this made it a potential substrate for the production of single cell protein (Shahzad and Rajoka, 2011). Microbes such as yeast, bacteria, algae, fungi are the prime candidate for SCP production because of their high specific growth rate and biomass yield. The yeasts have the advantages of large size, low nucleic acid content, long history of use as food and ability to grow at low pH (Singh *et al.*, 2011).

MATERIALS AND METHODS

Isolation procedure

Yeast cultures were isolated from ripened fruits such as apple, lemon, orange, pomegranate and tomato and soil samples such as garden soil, dairy soil, compost, tea waste containing soil and curd. All samples collected from fruit cart and local area of village Sadra, Gandhinagar, Gujarat, India. The yeasts were enriched by inoculation of 5 ml or 5 g of sample in 250 ml Erlenmeyer flask containing 100 ml of Malt Extract Broth (MEB), with 0.1 g/l Chloramphenicol.

The incubation was performed at 37°C for 48h on shaker at 100 rpm. The yeast strains were isolated on Yeast Extract Glucose Chloramphenicol Agar plates (YGCA) by serial dilution of Malt Extract Broth. YGCA contained (g/l); Agar, 15.0; Glucose, 15.0; KH_2PO_4 , 5.2; NH_4Cl , 0.54; Yeast Extract, 0.5; and Chloramphenicol, 0.1. All plates were incubated at 37°C for 72h. Isolates were maintained on Potato Dextrose Agar (PDA) slants at 4°C.

Selection and identification of potent yeast strain for single cell protein production

Fermentation carried out using basal media (NH_4) $_2\text{SO}_4$ - 2.0 g; KH_2PO_4 - 1.0 g; $\text{MgSO}_4 \cdot 7\text{H}_2\text{O}$ - 0.5 g; NaCl - 0.1 g; CaCl_2 - 0.1 g; distilled water 1000 ml; pH – 5.5 which is supplied with 5% (5 g) potato waste. For fermentation 100ml medium added in 250ml Erlenmeyer flask and inoculate with yeast 5 per cent inoculum. Incubate all flasks at 37°C for 72h on shaker at 100 rpm. Protein concentration and biomass concentration (g/l) of all isolates showed in Table 1. On the basis of colonial, morphological and biochemical characteristics, identification of potent yeast strain was done. Biochemical identification was carried out at Supratech Laboratory, Ahmedabad, Gujrat, India.

Inoculum preparation

The isolated culture of yeast was dissolved in 2 ml sterile D/W, and streaked on freshly prepared PDA slants. The PDA slants were placed at 37°C for 24h to activate the yeasts. The slant covered with growth was suspended in 10 ml sterile D/W. The cell count was subjected to 10^6 cells/ml by adjusting absorbance maxima λ_{max} at 600 nm. At 600 nm 1 O.D = 10^6 cells/ml. 5 per cent of this suspension was used to inoculate 100 ml fermentation medium.

Fermentation procedure

Submerged fermentation was carried out in 250 ml cotton plugged Erlenmeyer flasks containing 100 ml medium.

Unoptimized media were prepared consisting of the basal media $(\text{NH}_4)_2\text{SO}_4$ - 2.0 g; KH_2PO_4 - 1.0 g; $\text{MgSO}_4 \cdot 7\text{H}_2\text{O}$ - 0.5 g; NaCl - 0.1 g; CaCl_2 - 0.1g; distilled water 1000 ml; pH – 5.5 free from glucose, but supplied with 5 per cent (5 g) substrate. The medium was distributed in Erlenmeyer flasks and sterilized at 121°C for 15 minute at 15 lbs pressure. After cooling suitable volume 5 per cent (5ml) inoculum was added. The flasks were incubated at 37°C at 100 rpm on rotatory shaker for 72h.

Selection of substrate

Different types of substrates such as banana peel, orange peel, orange pulp, corn stover, dairy waste water, tea waste, bagasse and sorghum were collected from fruit cart and local area of village Sadra, Gandhinagar, Gujarat, India. Amongst these all substrate, banana peel used for SCP production depending upon the higher amount of protein concentration obtained with isolated yeast (Figure 1). Protein production by all substrates using potent strain HSP₉ was checked under submerged fermentation.

Biomass Recovery

After complete incubation, fermentation broth was allowed to filtrate through mashline cloth and washed biomass containing substrate with 50 ml D/W. After filtration culture broth was centrifuged at 10,000 rpm for 10 minutes. The cell mass suspended in 10 ml distilled water allowed to dry in oven at 60°C. After drying, dry weight of biomass was measured quantitatively.

Optimization of physicochemical parameters for SCP production

Effect of pH on SCP production

This experiment was designed to determine best pH value that stimulates single cell protein production by yeast. The pH of the fermentation medium was varied from 4.5, 5.0, 5.5, 6.0, and 6.5. pH was adjusted by 1N HCl or 1N NaOH. The

fermentation medium was inoculated with 5 per cent (V/V) inoculum and all flasks were kept at 37°C at 100 rpm for 72h under submerged fermentation condition.

Effect of temperature on SCP production

During fermentation heat is evolved due to metabolic activity of yeast and thus temperature increase. This can be evaluated by incubation of fermentation medium having pH 5.5 and inoculated with 5 per cent (V/V) pure yeast culture at different temperature from 30°C to 45°C for 72h.

Effect of inoculum size on SCP production

The effect of different inoculum size on single cell protein production by yeast strain was investigated. The inoculum of *Candida krusei* at the concentration of 2, 4, 6, 8 and 10 per cent V/V to investigate optimum inoculum size. All flasks were incubated at 37°C for 72h.

Effect of substrate concentration on SCP production

This experiment performed to demonstrate maximum nutrition requirement of yeast to produce single cell protein in terms of substrate concentration. In this study substrate concentration was studied for 2, 4, 6, 8 and 10 per cent (W/V) with 100 ml fermentation medium to check its effect on SCP production for *Candida krusei* (HSP₉). The optimized pH, temperature, inoculum size and substrate concentration were kept constant for further study.

Effect of incubation period on SCP production

Optimization of incubation period was performed to determine the time required for the maximum SCP production. For this, 250 ml flasks contains fermentation medium with pH 5.5 were inoculated with 6 per cent (V/V) inoculum incubated at 37°C. SCP production was carried out at regular interval of 12 to 84h.

Effect of agitation speed on SCP production

Agitation allow good oxygen transfer rate and maintain homogenous environment in fermentation medium. So, in submerged fermentation, there was need to know the optimum agitation speed for SCP production. In this investigation, different agitation speed was used ranged between 80-160 rpm. Other optimized parameter kept constant.

Effect of ammonium sulfate $[(NH_4)_2SO_4]$ concentration on SCP production

The concentration of ammonium sulfate $[(NH_4)_2SO_4]$ was varied from 0.5 to 4.5g/l. The other medium components were kept constant.

Effect of nutrient supplementation on SCP production

Effect of organic nitrogen source on SCP production

Organic nitrogen such as peptone supplemented at different concentration 2 to 14g/l along with fermentation medium.

Effect of carbon source on SCP production

Effect of carbon source on SCP production was studied in form of sugar such as glucose. For this purpose, glucose added at different concentration 2 to 10g/l along with fermentation medium. All flasks inoculated and incubated as per previously optimized conditions.

Comparison between optimized and unoptimized condition

One flask was kept at unoptimized condition and another was kept with above all optimized condition such as pH (5.5), temperature (37°C), inoculum size (6%), substrate concentration (6%), Incubation time (72h), agitation speed (120 rpm), $(NH_4)_2SO_4$ concentration (4.5g/l).

Biomass analysis

Biomass analyzed for protein content by Folin phenol reagent (Lowry *et al.*, 1951), nitrogen by total Kjeldahl method, reducing sugar by Dinitrosalicylic acid (DNSA) method and amino acids by Ninhydrin method (Thimmaiah, 1999).

RESULTS AND DISCUSSION

In order to achieve the maximum SCP production by yeast cells, twelve yeast isolates were successfully isolated from ripened fruits and soil samples. Among twelve isolates, potent yeast strain was selected based on their efficiency to yield the maximum protein content. As illustrated in Table 1, the maximum protein producing yeast strain was identified as *Candida krusei* (HSP₉). Thus HSP₉ was used in all the subsequent studies.

Morphological characteristics of HSP₉ were studied by Gram's staining (Table 2) and colony characteristics were also studied, shown in Table 3. Further identification of HSP₉ was confirmed at Supra- Tech Laboratory, Ahmedabad, Gujrat, India. On the basis of VITECH 2 system 05.04, yeast culture was identified as *Candida krusei*. Results of various biochemical tests are given in Table 4. To reduce the cost for production of SCP, cheap and easily available raw materials should be used. Eight different substrate like banana peel, corn stover, dairy waste water, tea waste, bagasse, orange peel, orange pulp and sorghum used for SCP production, among them banana peel gave the highest protein content. Banana peel contains 13.5g/l carbohydrate which is determined by Anthrone's method (Figure 1).

Optimization of physicochemical parameters for SCP production

pH

It has been noted that pH value in the medium has great influence on protein production (Anupama and Ravindra, 2000; Gao *et al.* 2007). The results presented in the Figure 2 indicated that the potent yeast *Candida Krusei* (HSP₉) produced the highest protein content (4.52 g/l) at pH 5.5. Decreased and increased in pH range showed decreased in protein yield. In harmony with our finding, Soroush *et al.* (2013) recorded the maximum SCP

production at pH 5.5 from fish protein isolate wastage and ultra filtered cheese whey. Gao *et al.* (2007) recorded protein production with optimum pH 5.5 using *Candida tropical*. Dharumadurai *et al.* (2011) produced optimum protein production by *Saccharomyces cerevisiae* MTCC 463 and *Candida tropical* MTCC 14 under submerged fermentation at pH 5.5. From above reported work and our finding, it can be concluded that the slight acidic pH might be suitable for SCP production.

Temperature

Temperature is one of the most critical parameter that has to be controlled in bioprocess (Chi and Zhao, 2003; Gao *et al.*, 2007). As illustrated in Figure 3, protein content (4.84g/l) was noted the highest when they were cultivated at 37°C. At 42°C, protein content was decreased rapidly. Narayanan *et al.* (2012) reported that the *Saccharomyces cerevisiae* MTCC174 was able to grow at 37°C to 45°C and produced SCP using different fruit wastes. Bacha *et al.* (2011) reported the maximum protein concentration (5.29g/40g substrate) at 37°C using *Saccharomyces cerevisiae*. On the other hand, Moeini *et al.* (2004) produced SCP at 37°C by eleven different yeast strains (M₁ to M₁₁) and removed BOD from whey.

Inoculum size

One of the most important parameter is the optimization of inoculum size which affects the overall SCP production by interfering with substrate utilization rate. The obtained results illustrated in Figure 4 clearly showed that, the optimum culture density for SCP production was 6% V/V with the highest protein productivity (5.22g/l). Initially as inoculum size increases simultaneously increased the protein content, but at 8% V/V inoculum size, protein concentration decreased gradually. The reason behind this could be attributed to dissolve oxygen limitation and increasing

competition toward nutrients for metabolism and growth which ultimately affecting final product formation. Irfan *et al.* (2011) suggested that 7% (V/V) inoculum size suitable for the maximum protein yield. Yalcin and Ozbas (2008) suggested that inoculum size 5% (V/V) for the maximum *Saccharomyces cerevisiae* biomass production.

Substrate concentration

The rate of SCP production is well controlled by the available substrate concentration. Results regarding protein content of the biomass produced with different substrate level are illustrated in Figure 5. It was noted that with an increase in substrate concentration the protein content of the biomass increased and reached the maximum (4.73g/l) at 6% (W/V) substrate concentration. At 8 % (W/V) substrate concentration, fairly decreased in protein yield (4.20g/l) was noted. It might be due to the exhaustion of substrate concentration that might interfere oxygen transfer leading to limitation of dissolved oxygen for yeast growth. Gao *et al.* (2012) stated that, total protein was increased from 2.61 to 4.70g/l when the soy molasses concentration was increased from 1 to 7g/l. Results are compared with Ahmed *et al.* (2010), who used different concentration of corn stover ranging from 0 to 8% (W/V) and the maximum production was obtained at 6% (W/V) substrate concentration using *Arachniotus sp.* and *Candida utilis*.

Incubation period

Time course play a very critical role in yeast metabolic activities and growth. The results depicted in Figure 6 illustrated that the biomass yield of *Candida Krusei* (HSP₉) when grow on different period of time from 12 to 84h. Initially as time increases, the protein content also increased in ordered manner up to the maximum protein yield (5.0 g/l) at 72h. It was the indication of the

exponential phase of yeast metabolism and growth. After 72h incubation period, the protein yield decreased up to 4.53 g/l. It might be indication of declining phase of potent yeast due to nutritional starvation condition in fermentation medium. Consequently, the biomass yield of the *Candida krusei* were reported the maximum at 72h fermentation period by Ahmed *et al.* (2010), Yabaya *et al.* (2009) and Kurbanoglu (2001), who studied on biomass yield of the fermentation period and obtained maximum protein at 72h. Popa *et al.* (2004) studied yeast strains biomass production capacity and physical, chemical characterization of the biomass and the production was recorded the maximum after 72h incubation period.

Agitation speed

Agitation allow good oxygen transfer rate and maintains homogenous environment into the fermentation medium. In a study, the maximum growth rate and protein productivity (8.76g/l) were realized at an agitation speed of 120 rpm at 37°C throughout the fermentation period of 72h. Decreasing and increasing in the agitation speed showed simultaneous decreased in protein productivity (Figure 7). Shahzad and Rajoka (2011) recorded the maximum protein yield at 120 rpm using *Aspergillus terreus*. Singh *et al.* (2011) grown *Humicola sp.* and *Saccharomyces cerevisiae* at 120 rpm for SCP production.

Ammonium sulfate [(NH₄)₂SO₄] Concentration

Nitrogen content in the medium is of extreme importance to protein yield. Medium containing (NH₄)₂SO₄ at 4.0g/l concentration was highly stimulated SCP production to yield the maximum protein concentration (11.64g/l) (Figure 8). Narayanan *et al.* (2012) noted the maximum protein concentration (11g/l) with the same nitrogen source at 4.5g/l by *Saccharomyces cerevisiae*. Amount of SCP production can

be improved with addition of ammonium sulfate as nitrogen supplementation at concentration 5g/l Gao *et al.* (2012). Gad *et al.* (2010) produced 7.8g/l protein with supplementation of (NH₄)₂SO₄ as inorganic nitrogen source. Jiru (2009) produced the maximum SCP at 5g/l ammonium sulphate concentration.

Nutrient supplementation on SCP production

Organic nitrogen source

Due to the structural properties of protein, the nitrogen source is one of important factor during the synthesis of protein by microorganisms (Taran and Bakhtiyari, 2012). The maximum protein yield (24.30g/l) was obtained at 10g/l peptone concentration. The results depicted in Figure 9 showed the effect of peptone concentration on SCP production. Protein content in *Candida Krusei* (HSP₉) was increased when the peptone concentration increased from 2.0 to 10g/l, but at 12g/l peptone concentration, the protein content decreased. These results indicated that medium supplemented along with peptone gave higher protein yield compared to only basal medium supplemented with banana peel. Gao *et al.* (2012) used peptone as a nitrogen source for SCP production using *Phanerochate chrysosporium* from *Oputia ficus indica* waste. Rosma and Cheong (2007) suggested that increment in biomass and protein yield when medium supplemented with peptone as nitrogen source with pineapple waste as a substrate using *Candida utilis*.

Carbon source

Carbon source play an important role in the production of protein by microorganisms. The results depicted in Figure 10 shows the effect of glucose concentration on SCP production. The results showed that the protein content in *Candida Krusei* (HSP₉) was increased when the glucose concentration was increased

from 2.0 to 10g/l. The maximum protein yield 23.12g/l was obtained at concentration 10g/l. This indicated that medium supplementation with glucose showed positive effect on the growth of *Candida Krusei* (HSP₉). The biomass growth was higher with increased in glucose concentration. Sankar *et al.* (2011) recorded the maximum protein yield at glucose concentration 3% W/V supplementation with banana peel. Poutou-Pinales *et al.* (2012) and Guimaraes *et al.* (2009) tested different concentrations of glucose (as carbon source), 1 to 8% (W/V) for SCP production in their work. The highest level of SCP production was obtained by growing the organism with glucose 8%. The results showed that glucose concentration affects the SCP production significantly. Protein content in the biomass was increased when the concentration of glucose was increased from 1% to 8% (W/V). The microorganism indeed produced SCP well from glucose as carbon source.

Comparison between optimized and unoptimized condition

To evaluate the effect of optimization on SCP production by *Candida krusei* (HSP₉) in submerged fermentation over unoptimized condition, the results of optimized and unoptimized condition were compared. It was found that at the end of complete optimization study, the maximum SCP production 11.54g/l was observed compared to unoptimized condition (4.5g/l). Therefore, it can be concluded that after optimization of fermentation condition, amount of protein yield increased as compared to before optimization.

Biomass analysis

The production of SCP by twelve different isolates was measured on the basis of weight of dry biomass (g/l) and protein content (g/l). The protein content of all isolates was measured by Folin phenol reagent (Lowry *et al.*, 1951) and obtained

results were showed in Table 1. Narayanan *et al.* (2012) reported the results of biomass yield (g/l) in banana peel as a substrate.

Biomass analysis of *Candida krusei* (HSP₉)

The biomass of *Candida krusei* obtained at the end of all optimized conditions were analyzed to determine crude protein content, total nitrogen content, reducing sugar and total amino acids and the results were presented in Table 5. Kandari and Gupta (2012) studied production of SCP from banana peel and produced 35.72 per cent crude protein and 7.47 per cent nitrogen.

Food or feed grade analysis of SCP

The SCP obtained from *Candida krusei* was analyzed for food or feed grade analysis at Jayshree Laboratory (Indian Govt. Approved), Rajkot. Biomass was analyzed for its rancidity, odor, insect, fungus infection, color and addition of adulterants and it is successfully passed through all above tests. It proved that the SCP was found to be food grade and is edible.

CONCLUSION

The potent yeast *Candida Krusei* (HSP₉) produced the highest protein content at a temperature of 37°C. The optimum culture density for SCP production was 6% V/V with the highest protein productivity. An increase in substrate concentration the protein content of the biomass increased and reached the maximum at 6% (W/V) substrate concentration. The protein content was the maximum at 72h incubation period. The maximum growth rate and protein productivity was realized at an agitation speed of 120 rpm at 37°C throughout the fermentation period of 72h. Medium containing (NH₄)₂SO₄ at 4.0g/l concentration was highly stimulated SCP production to yield the maximum protein concentration. The maximum protein yield (24.30g/l) was obtained at 10g/l peptone concentration. The protein content in

Candida Krusei (HSP₉) was increased when the glucose concentration was increased from 2.0 to 10g/l. It was found that at the end of complete optimization study, the maximum SCP production was observed compared to unoptimized condition.

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Table 1: Isolation and selection of potent yeast strain for SCP production

Code No.	Source of Isolate	Dry Biomass (g/L)	Protein Content (g/L)
HSP ₁	Apple	2.00	2.19
HSP ₂	Garden soil	3.00	3.29
HSP ₃	Compost	0.80	0.96
HSP ₄	Tea waste	3.00	4.46
HSP ₅	Tea waste	3.00	3.90
HSP ₆	Curd	1.20	2.17
HSP ₇	Dairy soil	1.80	3.25
HSP ₈	Orange	0.60	0.93
HSP ₉	Pomegranate	3.20	4.98
HSP ₁₀	Tomato	1.60	3.47
HSP ₁₁	Dairy soil	1.52	2.41
HSP ₁₂	Lemon	2.20	3.39

Table 2: Morphological characteristics of HSP₉ from Gram's staining

Characteristics	Observation
Gram's reaction	Gram positive
Size	Intermediate
Shape	Oval
Budding	Present
Arrangement	Single, Pair, Cluster

Table 3: Colonial characteristics of the HSP₉ on PDA plate

Characteristics	Observation
Size	Big
Shape	Round
Pigmentation	Creamish white
Texture	Smooth
Elevation	Convex
Margin	Erose
Opacity	Opaque

Table 4: Biochemical test of HSP₉

Sr. No.	Test	Result	Sr. No.	Test	Result
1	L-Lysine-ARYLAMIDASE	-Ve	24	L-SORBOSE assimilation	-Ve
2	L-MALATE assimilation	+Ve	25	L-RHAMNOSE assimilation	-Ve
3	Leucine-ARYLAMIDASE	+Ve	26	XYLITOL assimilation	-Ve
4	ARGININ GP	-Ve	27	D-SORBITOL assimilation	-Ve
5	ERYTHRITOL assimilation	-Ve	28	Saccharose/Sucrose Assimilation	-Ve
6	GLYCEROL assimilation	+Ve	29	UREASE	-Ve
7	Tyrosine ARYLAMIDASE	-Ve	30	ALPHA-GLUCOSIDASE	-Ve
8	Beta-n-acetylGlucosaminidase	-Ve	31	D-TURANOSE assimilation	-Ve
9	ARBUTINE assimilation	-Ve	32	D-TREHALOSE assimilation	-Ve
10	AMYGDALINE assimilation	-Ve	33	NITRATE assimilation	-Ve
11	D-GALACTOSE assimilation	-Ve	34	L-ARABINOSE assimilation	-Ve
12	GENTIOBIOSE assimilation	-Ve	35	D-GALACTURONATE assimilation	-Ve
13	D-GLUCOSE assimilation	+Ve	36	ESCULIN hydrolyse	-Ve
14	LACTOSE assimilation	-Ve	37	L-GLUTAMATE assimilation	+Ve
15	Methyl-A-D-Glucopyranoside assimilation	-Ve	38	D-XYLOSE assimilation	-Ve
16	D-CELLOBIOSE assimilation	-Ve	39	DL-LACTATE assimilation	+Ve
17	Gamma-glutamyl Transferase	-Ve	40	ACETATE assimilation	+Ve
18	D-MALTOSE assimilation	-Ve	41	CITRATE (SODIUM) assimilation	-Ve
19	D-RAFFINOSE assimilation	-Ve	42	GLUCURONATE assimilation	-Ve
20	PNP-N-acetyl-BD-galactosaminidase 1	-Ve	43	L-PROLINE assimilation	+Ve
21	D-MANNOSE assimilation	+Ve	44	2-keto-d-gluconate assimilation	-Ve
22	D-MELIBIOSE assimilation	-Ve	45	N-acetyl-glucosamine assimilation	+Ve
23	D-MELEZITOSE assimilation	-Ve	46	D-GLUCONATE assimilation	-Ve

Table 5: Result of biomass analysis by different analytical procedure

Yeast	Crude Protein (%)	Total N (%)	Reducing Sugar (µg/ml)	Total Amino Acid (µg/ml)
<i>Candida krusei</i>	33.50	7%	528.15	602.05

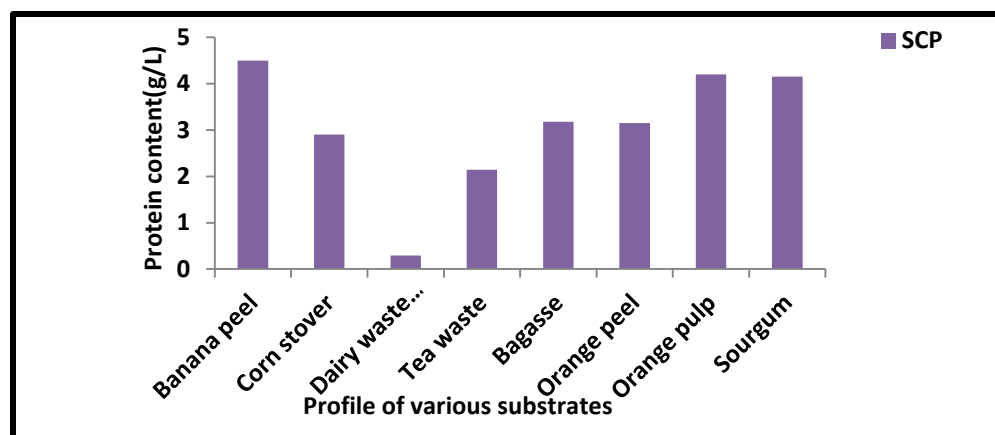


Fig. 1: Profile of various substrate on SCP production

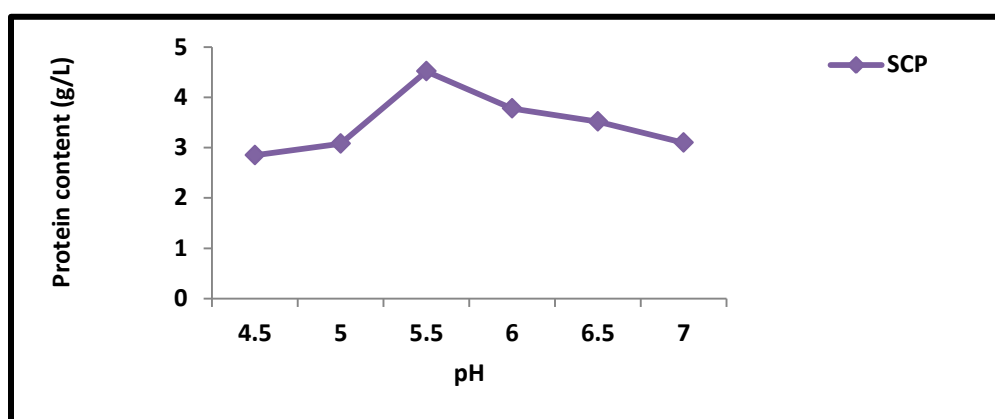


Fig. 2: Effect of pH on SCP production

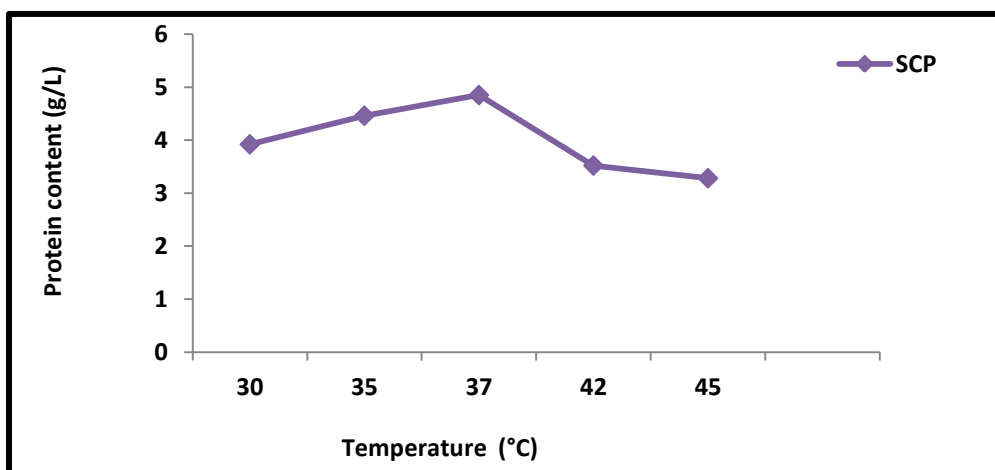


Fig. 3: Effect of temperature on SCP production

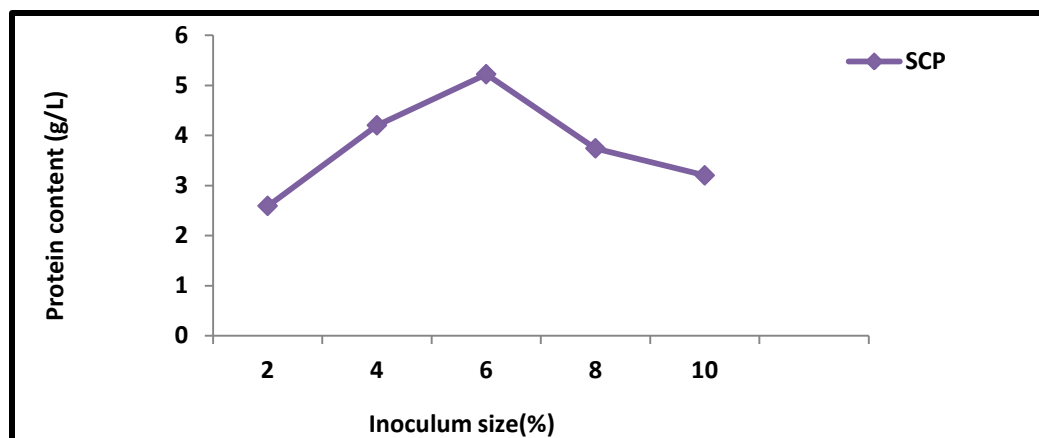


Fig. 4: Effect of inoculum size on SCP production

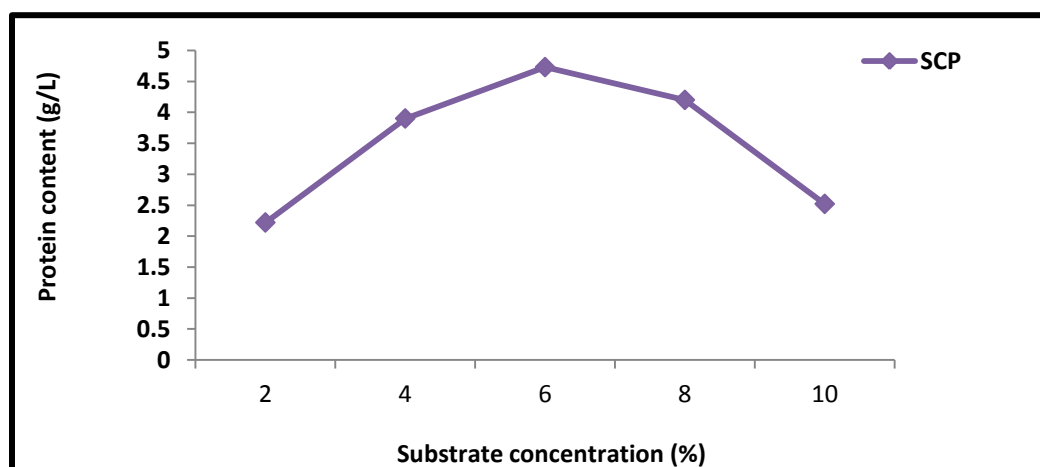


Fig. 5: Effect of substrate concentration on SCP production

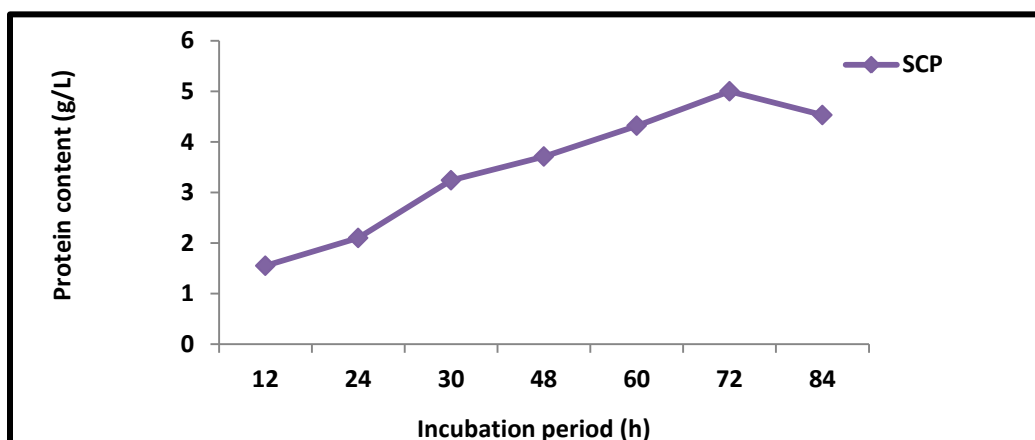


Fig. 6: Effect of incubation period on SCP production

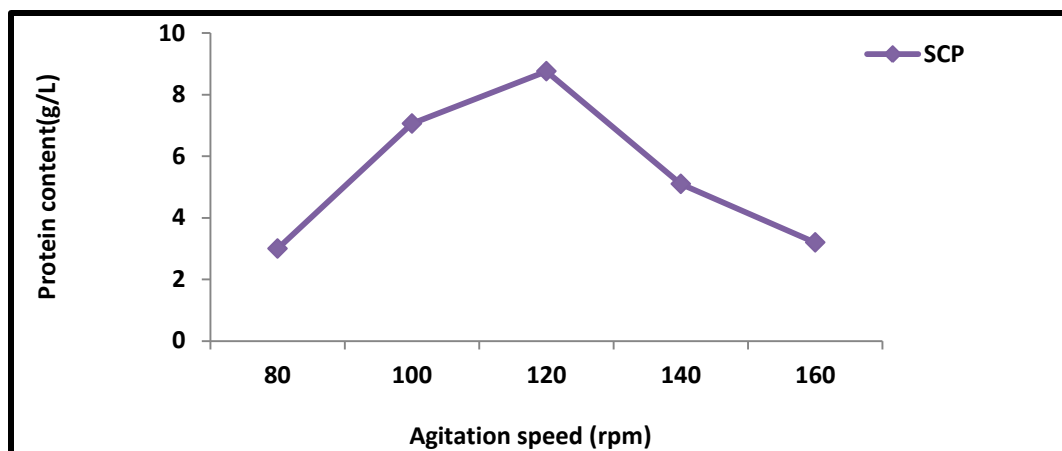


Fig. 7: Effect of agitation speed on SCP production

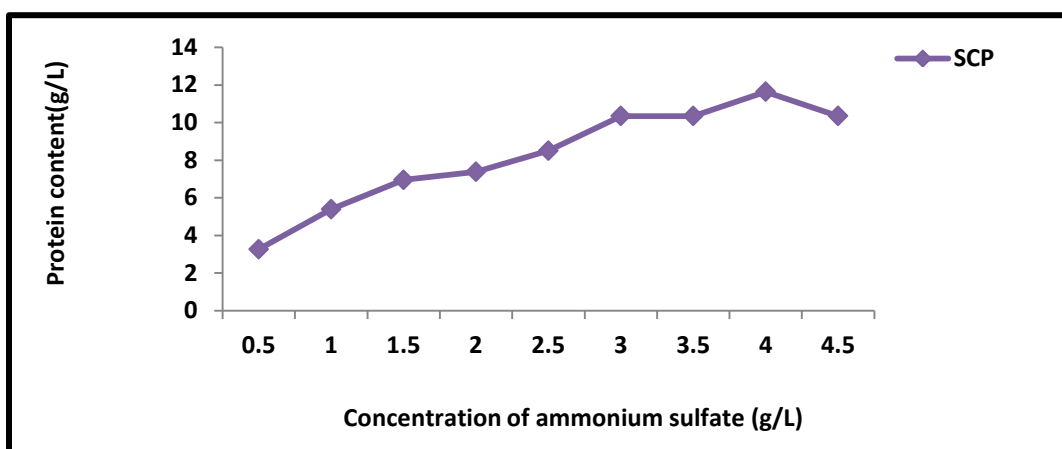


Fig. 8: Effect of ammonium sulfate on SCP production

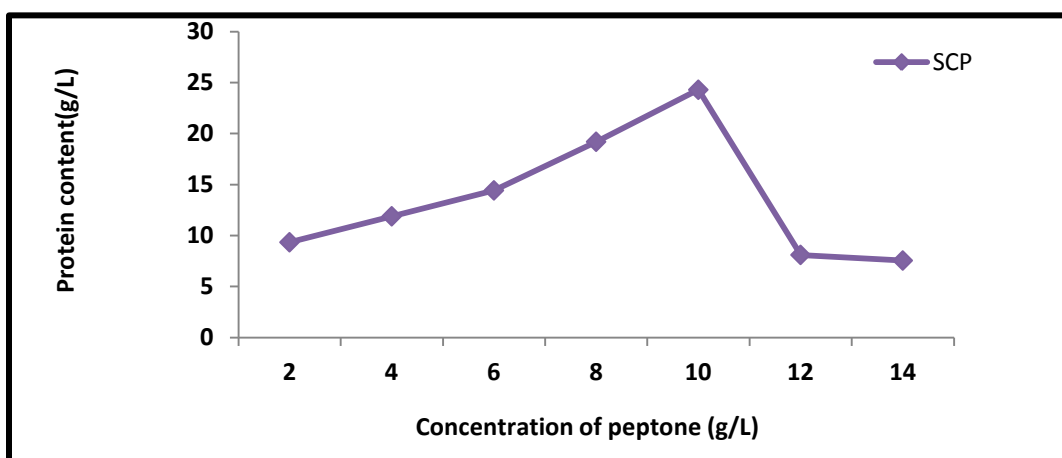


Fig. 9: Effect of peptone on SCP production

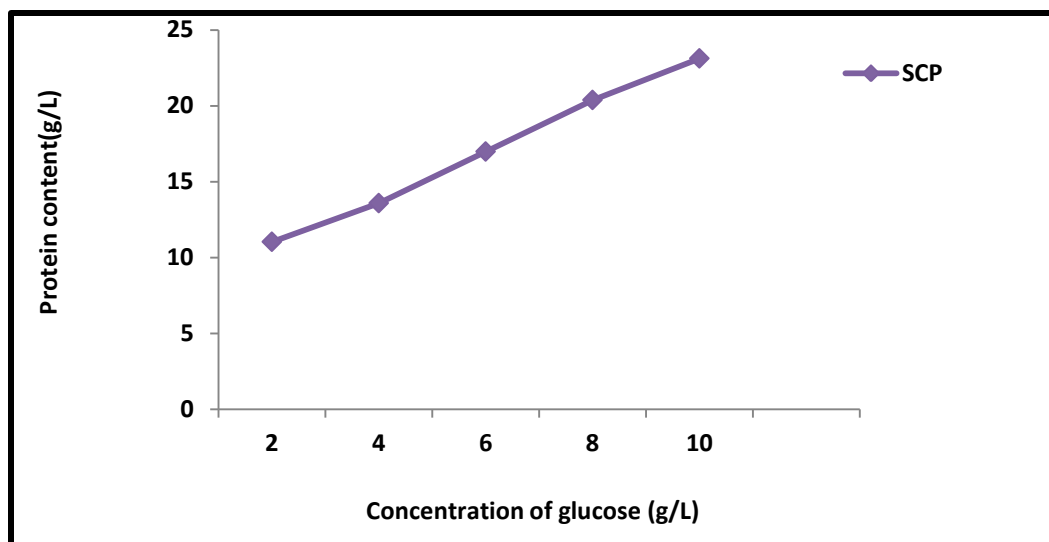


Fig. 10: Effect of glucose on SCP production

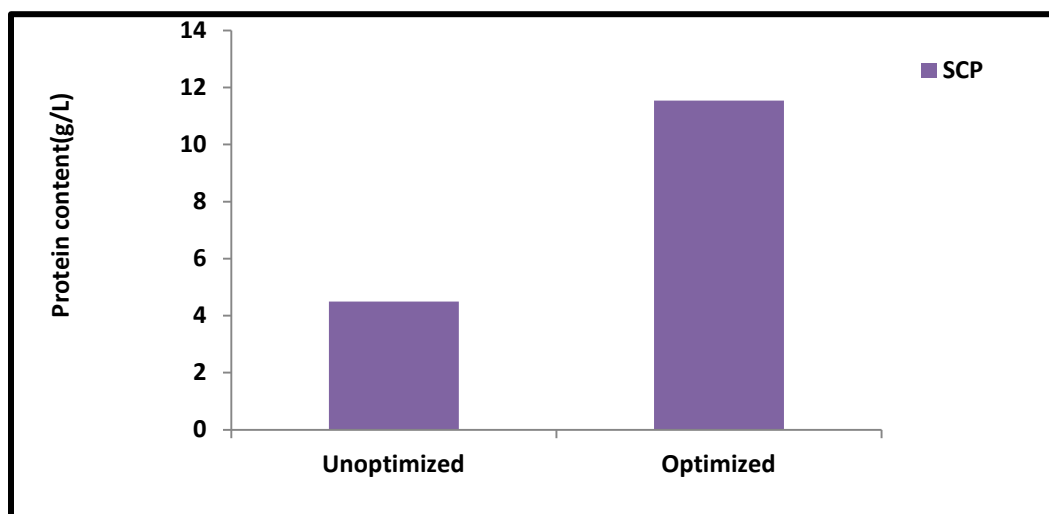


Fig. 11: Comparison between optimized and unoptimized condition

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