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Research Article

Biochemical studies on growth of single cell proteins with yeast extract supplement under varied biotic and abiotic conditions

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Abstract

The biological synthesis of SCP based on the yeast biomass *Saccharomyces cerevisiae* has gained considerable attention due to its widespread application in human food supplements. The wastewater obtained from cassava processing industry was used to produce Single Cell Protein by microbial fermentation method. The yeast biomass *Saccharomyces cerevisiae* was isolated from the soil sample by serial dilution and the subculture was done by yeast potato dextrose media. The inoculums were maintained by using MYGP media. Wastewater of cassava and the nutrients of cassava were mixed together for production of media preparation. The different variable parameters such as carbon source, nitrogen source, interaction time, temperature and pH were studied to help obtain maximum yield of SCP of the current experiment. The crude protein was determined by Lowry's method and growth of the yeast was measured by dried cell weight technique.

Keywords: cassava wastewater, *Saccharomyces cerevisiae,* MYPD media, starch, India.

Introduction

Proteins are macromolecules made up of amino acids which are highly essential for the existence of the living system [1]. The Single Cell Protein is used as the protein source in animal feed and protein rich food for humans. The mixed protein derived from the unicellular microbial biomass grown on biological waste has been used for the production of Single Cell Protein extensively in industry [2]. Many raw materials used for the production of Single Cell Protein have been considered as the carbon and other energy sources. The high protein content present in Single Cell Protein makes its use attractive for aquaculture, such as shrimp farming, prawns, shellfish, salmon, etc. [3].

Research on Single Cell Protein lies partially in the need for more protein and partially in commercial interest. It acts as the substitution for the conventional protein supplements used mainly in livestock feed [4, 5]. The production of Single Cell Protein assumes special significance on account of its nutritional and physiological function [6]. Biomass from waste is mainly used for the production of Single Cell Protein industrially by using microbial biomass including cassava waste and other agricultural wastes due to the presence of carbon and nitrogen sources for the substantial growth of the microbial biomass. Industrial work has been undertaken on the acid hydrolysis of shrimp-shell waste to directly produce protein and indirectly produce Single Cell Protein through a bioconversion process [7]. The main method used industrially for the production of Single Cell Protein has been microbial fermentation in which microorganisms having high protein content were used [8]. Microorganisms such as algae, yeast and bacteria give intensive growth, with optimum food supply under favourable conditions because bacteria are able to double within 0.5-2hr, yeast in 1-3hr and algae within 2-6hr [9].

The genetics and chemical composition of these microorganisms can be altered with relative ease because of rapid succession of generation. Single Cell Protein can be produced from cheap waste raw materials available in large quantities which minimizes environmental pollution [10]. Recent research has focused on the production of Single Cell Protein from carbohydrate sources [11] and plant origin liquid waste [12, 13].

The various groups of microorganisms that have been considered for food or feed use are algae, bacteria, yeast and moulds [14, 15]. The dried cells of these organisms are collectively referred to as "single cell protein". The term was coined by C.L. Wilson at the Massachusetts Institute of Technology in 1966.

Single Cell Protein can be produced by a number of different substrates, often this is done to reduce biological oxygen demand of the effluent streams leaving various types of agricultural processing plants. The various substrates which can be used as the common material for the production of various types of Single Cell Protein includes whey [16], orange peel residue, sweet orange residue, sugarcane bagasse, paper mill waste [17], rice husk, wheat straw residue, cassava waste, sugar beet pulp, coconut waste, grape waste, mango waste, etc. [18, 19]. Different types of algae such as chlorella, scenedesmus and spirulina are commonly used for the production of Single Cell Protein. They use carbon dioxide and sunlight as substrate. Algal SCP has about 60% crude protein which is generally good in amino acid composition except for deficiency in sulphur containing amino acid.

Thermo tolerant filamentous fungi have been used to produce SCP mainly from polysaccharide hydrolysates [3, 20] such as starch hydrolysates, sulphite liquor from wood pulp industries etc. [21, 22, 23]. They were found to have crude protein content of 50-55%. Members of the yeast family, such as *Saccharomyces*, *Candida* and *Torulopsis* have been widely studied for SCP production [24]. The SCP has 55-60% crude protein, which has good amino acid balance except for a deficiency in sulphur containing amino acid [25, 26].

A large number of bacterial species have been evaluated for SCP production, such as *Methylophilus* using methanol SCP and was found to have a very high crude protein content over 80%, with good amino acid composition. The economic feasibility of SCP will be dictated by possible applications in competition with comparable existing products. SCP is rich in protein and can be stored and shipped over a long distance. SCP does not create imbalance in natural ecosystems. No novel synthetic compound is produced and the technology, being based on recycling, is pollution free. In developing

SCP processes new technical solutions for other related technologies have been discovered e.g. in wastewater treatment, production of alcohol and other metabolites, enzyme technology and nutritional sciences. [13, 26, 27].

Materials and Methods

Isolation of yeast

Yeast is the heterogeneous group of fungi that superficially appear to be homogenious. The majority of ascomycetous and basidomycetous yeasts isolated by laboratories go unrecognized because most of them are heterothallic. Yeast like fungi reproduce asexually. The identification of these fungi is based upon a combination of morphological and biochemical criteria. Morphology is primarily used to establish the genera, where as biochemical assimilations are used to differentiate the various species.

Isolation technique for mixed cultures

All the initially isolated yeasts of the current experiment were contaminated in the mixed culture. The purity of isolated yeast was done by direct mounts and subsequent streaking through colony isolation. Pure cultures were used for assimilation procedure of the current experiment. Spatial isolation of streaking subcultures was considered for the purification of the yeast. The additional techniques used for the purification of yeast are listed below.

Serial dilution method

The serial dilution of the microorganism under experimentation was done by serial dilution method in liquid medium. The different dilutions considered for the current experiment were 1ml of the medium to 9ml of the sterile water for 1:10 dilution, 1ml of the 1:10 dilution to 9ml of the water for 1:100 dilutions and so on. The number of yeast per millilitre of the fluid was reduced by 9/10 in each dilution. Subsequent dilutions were made in ratios of 1:1000, 1:10, 000, 1:1, 00, 000, 1:1,000,000 or even 1:1:10,000,000. The original culture contained an extremely large number of organisms.

From each dilution of the current experiment usually with 1:100,1ml of the culture was transferred to a sterile agar plate. Spread plate methodology was used for the transfer of the microorganism under experimentation. All the experiments were done under purely aseptic conditions. In the spread plate technique the sample was first placed on the center of the solid, cooled agar medium in the sterile petriplate. The sample was then spread evenly over the medium surface with a sterile, blend glass rod. The colonies developed on the surface of the agar were observed after incubation.

Identification of yeast

Principle criteria and tests for identifying yeast culture characteristics

Direct mounts

Direct mounts were made in order to study yeast morphology microscopically and to determine purity of the isolates.

Lacto phenol blue staining

The fungal suspension was prepared with lacto phenol cotton blue stain. A small drop of lacto phenol was placed on the clean glass microscope slide and a small portion of the yeast colony formed was removed and placed into drop of the lacto phenol. A clean cover glass was placed over the suspended cells and observed microscopically. The suspension was covered with the cover slip and the cover

glass was sealed with fingernail polish to temporality preserve the mount. The whole setup was viewed under microscope as shown in Figure 1.

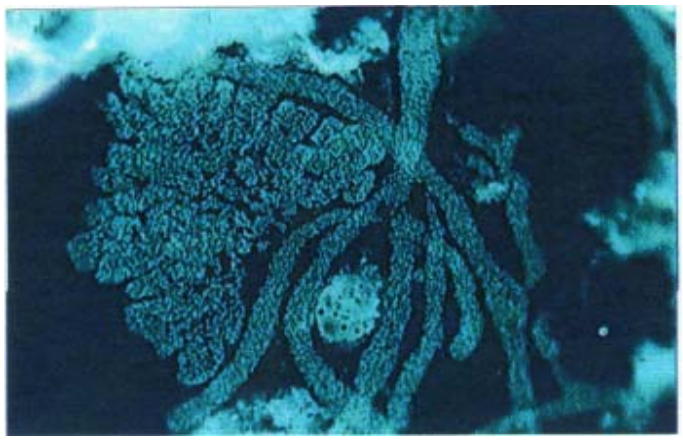

Figure 1. Microscopic View of Yeast Cultured after Lacto Phenol Test.

Sub culturing
Initially YPD media was prepared aseptically and filled in two test-tubes each having known volume in ml of the YPD media and the slants were made by allowing the medium to solidify after the particular interval of time. Then the isolated sample was inoculated in the solidified slant and kept in the test-tube with solidified sub cultured media inside the incubator at room temperature for one day.

Inoculum maintenance
The inoculum maintenance was done by keeping the test tubes inside a refrigerator in MYGP media at 4°C for future use.

Optimisation of culture media
Different media, such as sucrose, glucose and cassava were optimized for different variable parameters such as pH, temperatures, carbon source and nitrogen source. Finally cassava media was found to be the media with maximum growth.

pH
For studying the amount of protein content in the broth culture of cassava nutrients and wastewater of cassava was optimized at pH 6, 7, 8.

Temperature
The cassava processing wastewater and cassava were mixed well and after inoculation were kept at room temperature and also in the rotary shake incubator at 37°C.

Carbon source
For determining the best biomass yield in different carbon sources such as sucrose, glucose, maltose these were dissolved separately in cassava nutrient media while maintaining pH at 6 and then sterilized and placed in the rotary shaker incubator for 48 hrs.

Nitrogen source
Except for yeast extract and urea, all other chemicals of cassava nutrient medium were dissolved separately in peptone, sodium nitrate, beef extract, ammonium sulphate, maintaining pH at 6 and then inoculated with *Saccharomyces cerevisiae*, all the flasks being placed in rotary shaker incubator for 48hrs.

Preparation of production media
Wastewater obtained from cassava processing industry was taken and sterilized then the nutrient for the cassava media was also taken and sterilized. After sterilization both cassava wastewater and nutrient for cassava media were cooled and mixed properly.

Inoculation
In this process cassava ingredients were prepared and dissolved in cassava wastewater. Then both media were inoculated inside the laminar air flow using the culture slants previously maintained at 4°C. All the flasks were kept in the rotary shaker incubator at 37°C or room temperature for the growth of the yeast.

Mass production
Inoculum preparation for fermentation
20 ml of YPD media was taken and maintained at pH 6 before autoclaving. Then inoculation was done in broth media and kept at room temperature.

Bulk media preparation
One litre of wastewater obtained from the cassava processing industry was taken and sterilized inside the fermenter after autoclaving and cooled for 30 minutes.

Fermentation
A batch fermenter was used for the production of SCP from wastewater of cassava. The experiment set consisted of the fermenter, the air supply and the computer based data acquisition in control system. The fermenter and all accessories were chemically sterilized 2% potassium meta bisulphate solution and then washed with hot water for several times. The reactor was then filled with one litre of cassava wastewater and immediately inoculated with 20ml of the inoculum. Cell suspensions were prepared from 24hr old cultures in sterile media and transferred aseptically to the one litre flask containing cassava wastewater and pH of the medium was adjusted at 6 before autoclaving. The pH was maintained at 6 with the addition of HCL and NaOH using computer based pH measurement and control system. Dissolved oxygen and temperature were continuously monitored and the reactor was operated at 2vvp, at mixing speed of 300rpm. The samples were taken after 48hrs for maximum yield of biomass.

Isolation of single cell protein
10 ml of the solution was fermented and after that centrifuged at 6000rpm for 20 minutes. Then the supernatant was collected.

Purification methods
The supernatant obtained containing enzyme lipase was subjected to purification. The main steps for purification under the current experimentation are listed below.

Ammonium sulphate precipitation or salting out
Ammonium sulphate of 40% w/v was added to the cell free supernatant and was stirred for 4 hrs.The procedure was done at 4°C. The precipitate obtained was allowed to stand for 2 hrs and then collected by centrifugation at 15000 rpm for 20 minutes. The pellet obtained was dissolved in 2ml of 2mM glycine NaOH buffer, pH at 11 and protease activity was assayed.

Dialysis
Preparation of dialysis tubing
The dialysis tube of required length was boiled once in distilled water, containing a pinch of sodium aside and EDTA and then thrice distilled water.

One end of the dialysis tube was tied with thread. The tube was then filled with the protein solution to be purified. The sealed tubing was suspended in about 250ml of 25mM glycine NaOH buffer at pH 11.0. The setup was continuously stirred by means of a magnetic stirrer and the temperature was maintained at 4°C glycine NaOH buffer, pH 11.0 and was changed 4 times at an interval of 2 hrs.

Dialysis was done extensively until the protein concentrate was obtained and its protease activity was assayed. The protein concentrate was then subjected to ion exchange chromatography.

Ion –exchange chromatography
In this method, protein in the solution with a low ionic strength was added to the column. The protein interacted with the beads via electrostatic interaction. In anion exchange chromatography, the matrix carried out a net positive charge which interacts with negatively charged protein strongly. The removal of the protein from the column was achieved by washing the column with a buffer of decreasing pH. As the pH lowers, the charge of the protein is reduced, leading to the weaker interaction with positively charged matrix. In cation exchange chromatography the matrix was negatively charged and it interacts with positively charged proteins. Elution was again achieved by a gradient of ionic strength or by an increasing pH gradient. The method relied on having two pumps which can control the mixing of low and high ionic strength to form the buffer gradient that washes the column. Seperation still depended upon “theoretical plate” model because the protein is bound in a sharp band at the start of the column. As it was eluted it begins to pass down the column, still determined by its ability to re-associate with the stationary phase. The separation was achieved by two methods such as binding strength of protein at a given ionic strength and partition constant as it passed down the column after its initial displacement from the start of the column.

Activation of the column
The column material was soaked in distilled water for 1-2 hrs in a beaker. The material was then transferred into 50ml of 0.01M NaOH and was allowed to stand for 30 minutes. The above procedure was repeated twice. The matrix material was washed 3-4 times in distilled water for 30 minutes. The material was then transferred to 50ml 0.1N Hcl and was allowed to stand for 30 minutes. The above step was repeated twice .The material was washed with distilled water 3-4 times for duration of 30 minutes.

After washing the matrix material - DEAE.Sephadex was transferred to the glass column and was equilibrated with citrate phosphate buffer with pH of 6.2.

Fractionation of dialyzed protein
The dialysis protein was subjected to exchanger, DEAE- Sephadex column. The adsorbed protease was eluted by linear gradient of NaCl from zero to 250mM in the same buffer. Protease activity was assayed in each 1.0 ml of the 4ml h. The number of fractions obtained was 11. In the final step, the active fractions were pooled, concentrated by polyethylene glycol and dialyzed against citrate phosphate buffer, pH at 6.2, overnight.

SDS –PAGE
The anionic detergent Sodium Dodecyl Sulphate (SDS) dissociates proteins in their constituent polypeptide chains. Monomeric SDS binds tightly to most of the proteins at about 1.4 mg SDS/mg protein. Combined treatment with disulphide reducing agent completely unfolds the protein.

Polyacryl amide gel electrophoresis in the presence of SDS separates the polypeptide chains according to molecular weight. Thus molecular weight of the polypeptide chains of a given protein can be determined by comparing their electrophoretic mobility on SDS gels to the mobility of marker proteins with polypeptide chains of known molecular weight.

The most popular electrophoresis method is SDS-PAGE system developed by Laemmli. This is a discontinuous system consisting of two gels: Stacking gel. The samples are prepared in low conductivity buffer (60mM Tris-Cl, pH=6.8) and are loaded between the higher conductivity electrode buffer (25mM Tris,192mM glycine, pH=8.3) and the stacking gel (125mM Tris-Cl, pH=6.8). When the power is applied, a moving boundary region is rapidly formed with the highly mobile chloride ions in front and relatively slow glycinate ions in the rear. A localized high voltage gradient forms between the fronts, causing the SDS-protein complex to condense into a very narrow region and migrate between the chloride and glycinate phases. The total amount of the SDS has a considerable influence on the resolution. High concentrations of SDS can lead to broadening and spreading of the protein bands. At the interface of the stacking and separating gel the SDS-protein complex experiences a sharp increase in retardation due to their restrictive pore size of the resolving gel. The glycinate ions overtake the proteins, which then move in the space of uniform pH formed by the Tris and the glycine.

Separating gel
The glass plates, spacers and comb were cleaned with detergent and rinsed with water and dried. The glass plates were wiped with ethanol and were then sandwiched, depending on the gel system used.

The separating gel solution was prepared, except ammonium per sulphate and TEMED, in a vacuum flask. It was then de-aerated for 1 minute under vacuum. After adding the ammonium per sulphate and TEMED were mixed with the solution gently. With the help of the pipette the separating gel solution was filled between the glass plates sandwiched along the edge of the spacers, until the height of the solution was 1.0cm below the comb. Immediately the solution was overlayed with water saturated 2-utanol or isopropanol to exclude air and to obtain an even interface between the gels. The gels were allowed to polymerize for 45 minutes. The gel was polymerized when sharp interface was viewed below to overlay.

Stacking gel (5ml)
The stacking gel was prepared just before using the gel to maintain the ion discontinuities at the interface between the two gels. The ingredients were mixed and de-aerated before adding the ammonium per sulphate and TEMED.

The solution was removed from the top of the gel, rinsed with water and the area above the gel carefully with the filter paper was dry yield. The stacking gel solution was filled on the top of the separating gel. The well forming comb was placed in position, being careful not to trap air bubbles under the teeth. Visible polymerization of the gel should occur within 20 min.

3 x sample buffer preparation: 3 ratio sample
The SDS-reducing sample buffer was prepared by adding 100µl 2-mercaptoethanol to each 0.9ml sample buffer. The sample (10µl) was diluted with an equal volume of SDS-reducing sample buffer. The samples were heated for 3 minutes at 95°C. Hydrophobic (membrane) proteins were incubated at 15 minutes for 37°C.

Assembling
The electrophoresis cell was assembled; tubing, clamps and comb were removed. The upper and lower reservoirs were filled with electrode. The samples were loaded into the wells in the stacking gel. The electrophoresis unit was connected to the power supply. The lower electrode was anode (+) and the upper was cathode (-). Electrophoresis was started with low voltage (70volts) and increased to higher (200volts) until the blue dye had reached the bottom of the gel. The 10X buffer was diluted as per requirement with water.

Destaining
The gel was soaked in excess of staining solution for 1hr. It was destained with solution overnight and observed for bands.

Protein estimation
Protein estimation was done to quantify the amount of protein present in the sample by two methods:-

Lowry's method
For protein estimation the culture was transferred into the eppendorf and centrifuged at 8000rpm for 12 minutes. Then the pellet was taken and dissolved in 1ml of the phosphate buffer for protein estimation. It was then analyzed by UV Spectroscopy.

Beer-Lamberts law
When the beam of monochromatic light passes through the solution containing absorbing material, the intensity of the light decreases as the light of the absorbing medium increases. It was then analyzed by UV Spectroscopy.

Procedure
0.1, 0.2, 0.3, 0.4, 0.5, 0.6, 0.7 and 1ml of the working standard were pipetted into a series of test-tubes. 0.1ml and 0.2ml of the sample extracts were pipetted into two other test-tubes. The volume was made to 1ml in all the test-tubes. A tube with 1ml of the water served as the blank. The volume was made up to 5ml by adding the solution containing florescent particles. The OD was measured at 280nm.

Lowry's method
The blue colour developed by the phosphomolybdic phosphor in the folin ciocalteau by the amino acid tyrosine and the tryptophan present in the protein plus the colour developed by the biuretic reaction of the protein with the alkaline cupric tartarate are measured by the Lowry's method.

Procedure
0.2, 0.3, 0.4, 0.5, 0.6, 0.7 and 1ml of the working standard were pipetted into a series of test-tubes. 0.1ml and 0.2ml of the sample extracts were pipetted into two other test-tubes. The volume was made to 1ml in all the test-tubes. A tube with 1ml of the water served as the blank. 5ml of the reagent C was added to each test-tube including the blank. It was mixed well and allowed to stand for 10 minutes. The 5ml of the reagent D was added to each tube including the blank. It was mixed well and incubated at room temperature in the dark for 30 minutes. Blue colour developed. The reading was taken at 660nm with a colorimeter.

Results and Discussion

Yeast microorganisms were extracted. Identification of the yeast was done by gram staining, lacto-phenol test. Optimization of media was done for pH, temperature, carbon source and nitrogen source and cassava media was selected as the production media. The yeast was inoculated in cassava media and then transferred to the fermenter. After two days SCP was extracted by centrifugation. The purification was carried out by salting out method, dialysis, ion exchange chromatography and finally by SDS PAGE. Protein estimation was done by spectroscopy and Lowry's method. Following are the results obtained.

Gram staining
The strain that appears as purple colour and rounded shape showing budding belongs to the yeast *Saccharomyces cerevisiae.*

Lacto phenol test
The cytoplasm was seen as a lightly stained blue region, forming a round shape and having budding. The microscopic view of the yeast culture after lacto phenol test was shown in Figure 1.

pH optimization
The optimum pH was found to be 6, as shown in Figure 2 and the OD values obtained at different pH levels are shown in Table 1.

Table 1. OD values obtained at different pH.

pH	Concentration	OD
5	0.1	0.35
	0.2	0.39
6*	0.1	0.46
	0.2	0.49
7	0.1	0.44
	0.2	0.41
8	0.1	0.41
	0.2	0.42

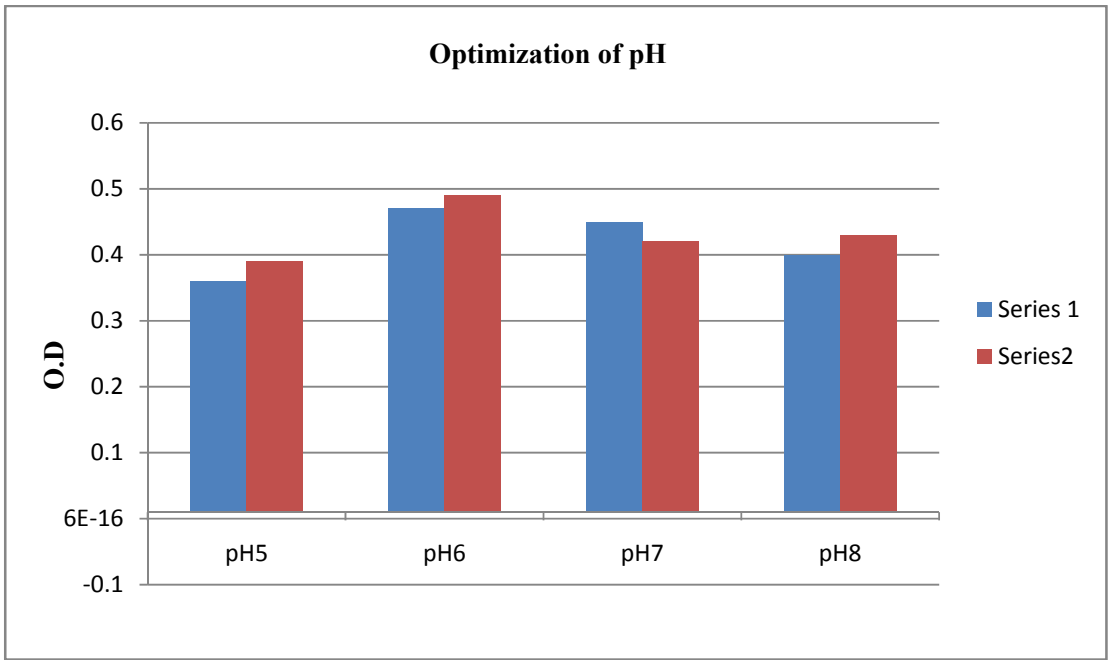

Figure 2. pH Optimization.

Temperature optimization

The optimum temperature was found to be 28°C, as shown in Figure 3 and the different OD values obtained at different pH are revealed in Table 2.

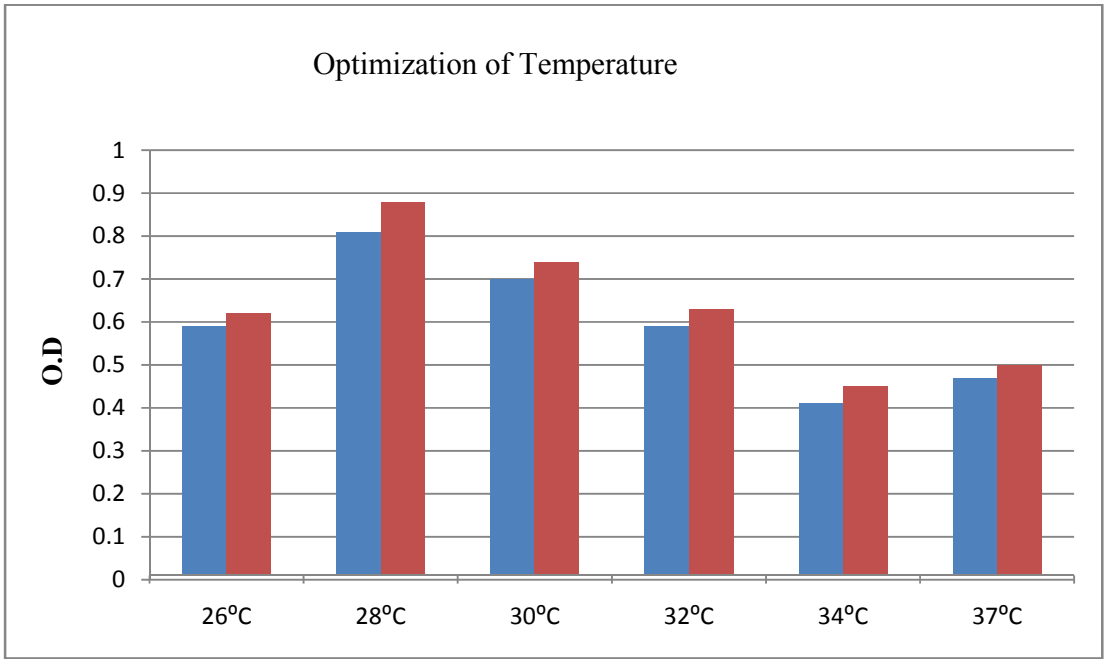

Figure 3. Temperature Optimization.

Table 2. OD values obtained at different temperature.

pH	Concentration	OD
26	0.1	0.59
	0.2	0.63
28*	0.1	0.81
	0.2	0.87
30	0.1	0.70
	0.2	0.74
32	0.1	0.58
	0.2	0.62
34	0.1	0.41

Optimization of carbon source

It can be seen from Figure 4 that cassava media was found to have maximum carbon content. Different OD values obtained for different carbon sources are shown in Table 3.

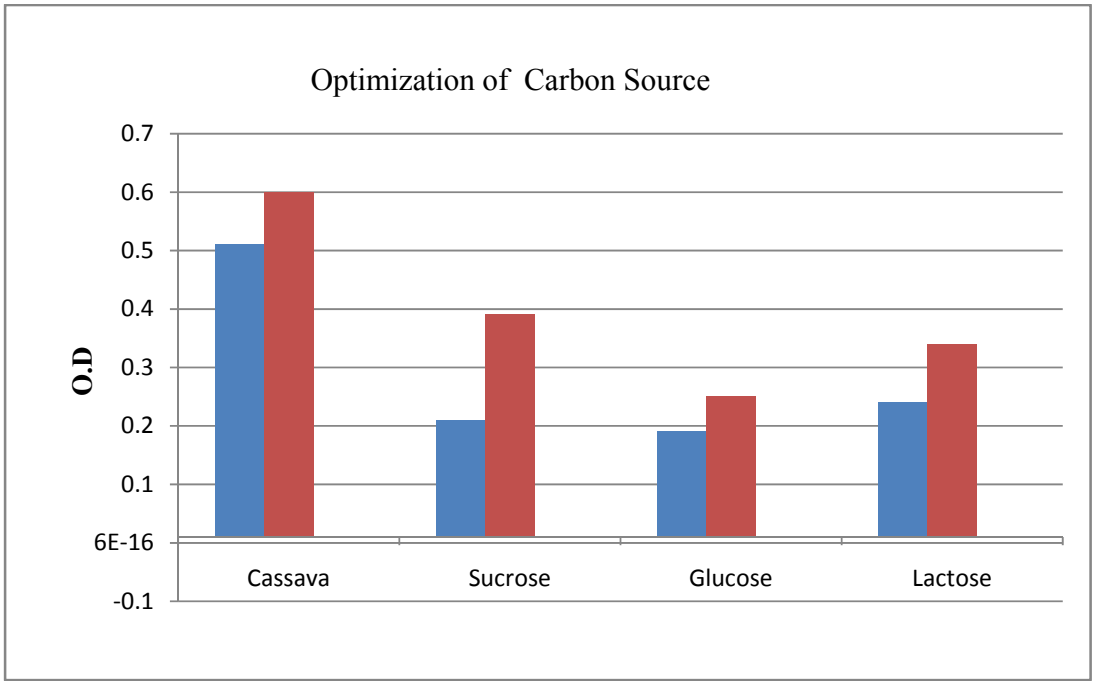

Figure 4. Carbon Source Optimization.

Table 3. OD values for different carbon sources.

pH	Concentration	OD
Cassava*	0.1	0.51
	0.2	0.60
Sucrose	0.1	0.21
	0.2	0.38
Glucose	0.1	0.19
	0.2	0.26
Lactose	0.1	0.23
	0.2	0.35

Optimization of nitrogen source

It can be concluded from Figure 5 that cassava media was found to have maximum carbon content. The different OD values obtained for different carbon sources are shown in Table 4.

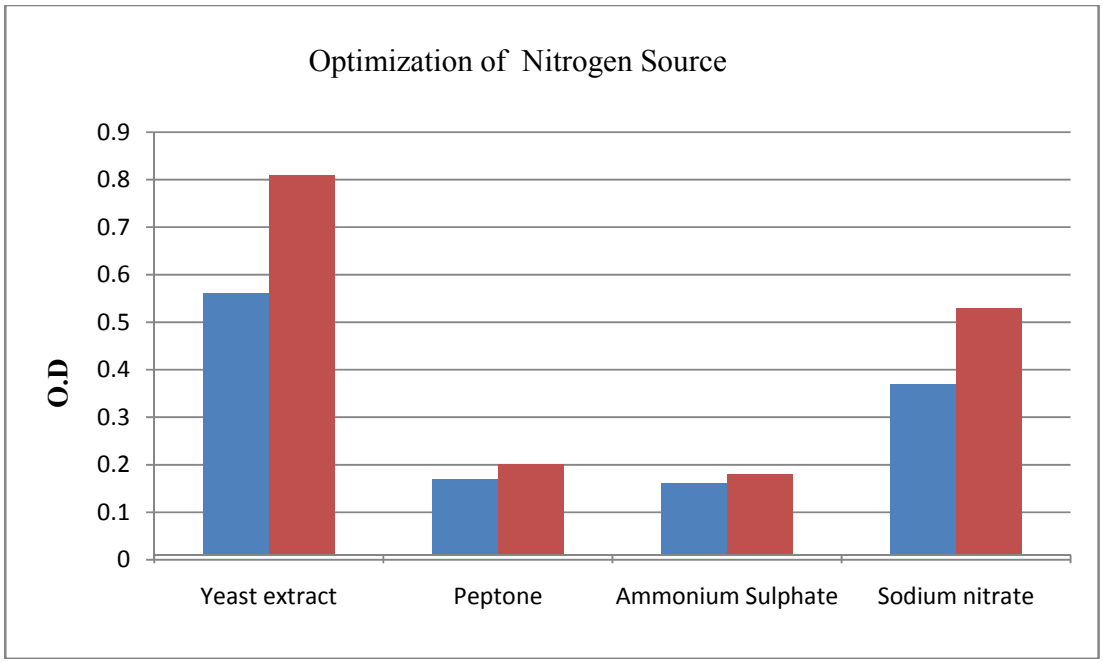

Figure 5. Nitrogen Source Optimization.

Table 4. OD values for different nitrogen sources.

pH	Concentration	OD
Yeast Extract*	0.1	0.56
	0.2	0.81
Peptone	0.1	0.18
	0.2	0.20
Ammonium Sulphate	0.1	0.16
	0.2	0.18
Sodium nitrate	0.1	0.36
	0.2	0.52

Protein estimation

Figure 6 shows UV spectro-photometric analysis by Beer Lamberts Law for protein samples at different concentrations. The samples having 5mMNaOH show maximum OD value at 280nm. The OD values obtained for different samples at 280nm are shown in Table 5.

Figure 7 shows UV spectro-photometric analysis by Lowry's method for protein samples at different concentrations. The samples having 5mMNaOH show maximum OD value at 280nm. The OD values obtained for different samples at 280nm are shown in Table 6.

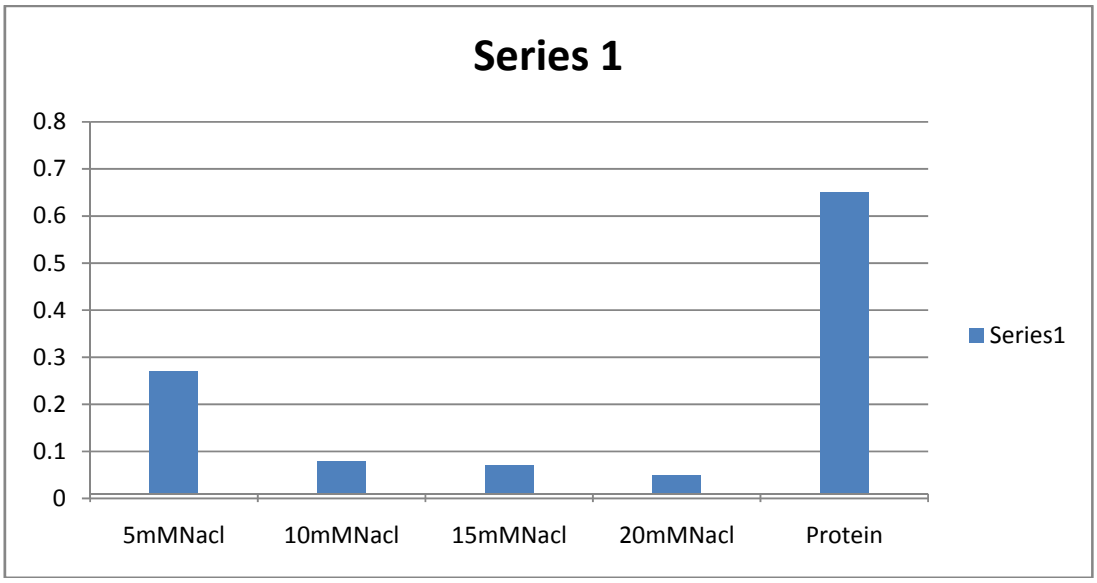

Figure 6. UV analysis of protein samples at different concentrations by Beer Lamberts Law.

Table 5. Readings obtained at different concentrations using Beer Lamberts Law.

S. No.	Samples	OD at 280nm
1	5mM Nacl	0.276
2	10mM Nacl	0.073
3	15mM Nacl	0.051
4	20mM Nacl	0.034
5	Protein	0.633

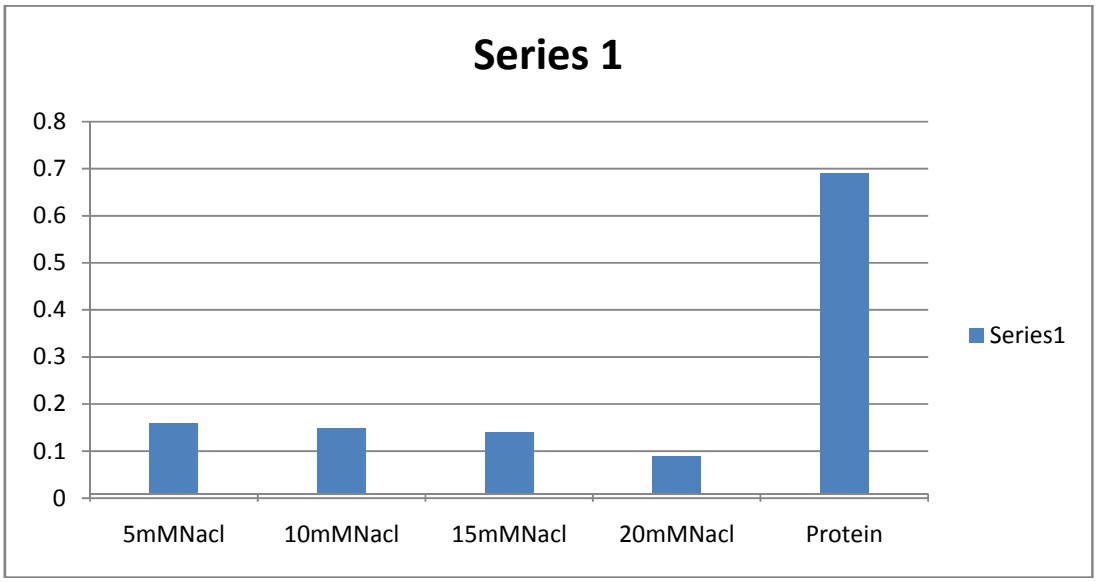

Figure 7. UV analysis of protein samples at different concentrations by Lowry's Method.

Table 6. Readings obtained at different concentrations using Lowry's Method.

S. No.	Samples	OD at 280nm
1	5mM Nacl	0.17
2	10mM Nacl	0.16
3	15mM Nacl	0.15
4	20mM Nacl	0.091
5	Protein	0.691

Ammonium sulphate precipitation or salting out

The precipitate was allowed to stand for two hours and then pellets were collected after centrifugation at 15000gm for 20 minutes.

Dialysis

Dialysis was undertaken and small molecular weight molecules were removed.

Ion exchange chromatography

Proteins are separated by ion exchange chromatography.

Figure 8. SDS-PAGE Electrophoresis.

SDS-PAGE

The different protein concentrations obtained was identified by SDS-PAGE run through electrophoresis are indicated by the dark bands shown in Figure 8. The molecular weight of the suspected SCP is 66KDa. The current experiment obtained ends at 60 and 52 KDa showing the SCP.

Conclusion

Cassava processing wastewater was found to be acidic. *Saccharomyces* species have indicated that limitation of nitrogen sources. The addition of different nitrogen sources exerted no noticeable effects on the yeast yields. No correlation was shown between cell mass yield and efficiency of the cells in producing proteins. Thus the medium containing yeast extracts supported best growth. The pH of the medium was maintained at 5, 6, 7 and 8 during the course of experiments. The optimum growth of *S. cerevisiae* was achieved at pH6. The SCP production from yeast was affected by temperature. The various temperature conditions were tested and it was found that room temperature gave the highest yield. As SCP production involved intracellular proteins, high speed agitation was required during fermentation for maximum propagation of yeasts. The dry yeast was taken for its content of crude protein. SCP from wastewater materials helps minimize environmental pollution as the waste stream

is converted to valuable products that can be used as protein sources in human food or animal feed. In the present study, the media was optimized for production of SCP. The optimum parameters obtained were:

pH - 6
Temperature - 28ºC
Carbon source - 0.6(cassava media)
Nitrogen Source - 0.82(yeast source)

Thus the protein content in the SCP was quantified. The protein was purified using salting out, dialysis, ion exchange chromatography methods and its high content was confirmed. SDS PAGE was done and the protein bands were obtained at 60 and 52 KDa showing SCPs.

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