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Studies on media optimization and reutilization of media

Yeturi Gayatri, Supriya Sarkar*

Dept. of Microbiology, St.Francis College for Women, Begumpet, Hyderabad - 500 016, (INDIA)

E-mail: yeturigayatri82@googlemail.com; supriya.sarkar4@gmail.com

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ABSTRACT

Media is an important substrate for the growth of different types of microorganisms. The most common growth media for microorganisms are nutrient broth and agar plates. The aim of the investigation was to reutilize the medium by supplementing the lost constituents, thereby reducing the investment on media. The protein and carbohydrate content decreased when the medium was reutilized for culturing proteolytic bacteria and fungi. The growth of the microorganisms was good when the media was resupplemented with lost nutrients such as protein and carbohydrate when compared with the media which was not resupplemented. The effect of media supplemented with deficient nutrients was studied on proteolytic bacteria and fungi, the growth of the microorganisms decreased after supplementation of the media when compared to growth on fresh medium.

KEYWORDS

Proteolytic microorganisms;
Media optimization;
Carbohydrate content;
Protein content.

INTRODUCTION

Culture medium is a liquid or gelatinous substance containing nutrients in which microorganisms or tissues are cultivated for scientific purposes. Media optimization is a method where the media is reused to minimize the investment on media in fermentation industries. Media composition affects the secretion of metabolites like extracellular enzymes[1-3] and polysaccharides[4,5]. The investment on media which are used for the production of different types of industrially important products is much and the product obtained will be in lesser amounts when compared with the media used. So to minimize the investment on media used, media optimization was carried out in the present study where the same media is reused for the growth of microorganisms and whether or not the same media can be used for reculturing microorganisms by supplementing the media with the deficient nutrients (carbohydrate and protein) is observed. The work was carried out on both proteolytic bacteria and proteolytic fungi.

MATERIALS AND METHODS

Soil sample was collected from tannery industry, Warangal.

Media

Nutrient agar medium and Nutrient broth each supplemented with 1% casein was used for the isolation of proteolytic bacteria from the different soil

FULL PAPER

samples. Similarly Asthana & Hawker's agar and broth medium each supplemented with 1% casein was used for the isolation of proteolytic fungi.

Screening

The soil sample was suspended in 100 ml sterile water. This solution was used as stock for serial dilutions. To prepare serial dilutions 1ml of soil sample was added to 10 ml of distilled water in one test tube. 5 test tubes of 9 ml distilled water each were taken and 1ml of the initial solution was transferred into successive test tubes to make 10^{-1} to 10^{-5} dilutions.

To isolate proteolytic bacteria 0.5 ml of 10^{-3} aliquot was spread plated on 5 nutrient agar plates supplemented with 1% casein under aseptic conditions. The plates were incubated at 37^0C for 48 hrs. After the incubation period distinct colonies with high zone of clearance were selected from each plate and were subcultured on slants to obtain pure cultures. For fungi 0.5 ml of 10^{-3} aliquot was spread plated on 5 Asthana & Hawkers medium agar plates supplemented with 1% casein and the plates were incubated at 28 ^{0}C for 5 days. After the incubation period the fungal mat was selected from each plate and was sub cultured onto slants to obtain pure cultures.

Determination of biomass

The biomass was determined by using formula:

Wet weight of filter paper heated at 161 °C - Dry weight of filter paper after filtering the culture broth before filteration (TABLE 1 and TABLE 2)

TABLE 1 : Biomass of bacteria

	Dry weight of filter paper(g)	After filteration (g)	Biomass (g)
Before inoculation	0.99	0.37	2 .71
After inoculation	2	4.6	2.6

TABLE 2 : Biomass of fungi

	Dry weight of filter paper(g)	After filteration (g)	Biomass (g)
Before inoculation	1.72	3.46	1.74
After inoculation	1.4	3.43	2.03

Preparation of inoculum

The nutrient broth and Asthana & Hawkers broth (250 ml) each supplemented with 1% casein were prepared and their protein and carbohydrate contents were estimated by Biuret method[6] and Anthrone method[7] respectively. The broths were inoculated with the pure culture and were incubated at 37°C for 48 hours (for bacteria) and 28°C for 5 days (for fungi).

Media harvest and estimation

After the incubation period the set of flasks were harvested on previously dried and weighed Whatman filter paper No. 42 and biomass was determined. The culture broth was then centrifuged at 5000 rpm for 10 minutes to remove any cell debris or cells. The supernatant was collected and used for protein and carbohydrate estimation.

Estimation of protein content

The protein content was estimated by Biuret method[6]. 1ml of the culture filtrate was taken and 3 ml of distilled water was added. To this 6 ml of Biuret reagent was added and mixed well on a cyclomixture. Then it was kept at 37°C for 10 min. The tubes were cooled to room temperature and the absorbance was read at 520 nm (green filter) against the reagent blank. (TABLE 3 and TABLE 4, Figure 1 and Figure 2).

TABLE 3 : Protein and carbohydrate content in initial broth and inoculated broth for bacteria

	Protein content O.D at 520nm	Concentration (µg/ml)	Carbohydrate content O.D at 630nm	Concentration (µg/ml)
Initial Broth	0.20	40	0.07	70
After noculation	0.11	22	0.058	50

TABLE 4 : Protein and carbohydrate content in initial broth and inoculated broth for fungi

	Protein content O.D at 520 nm	Concentration (µg/ml)	Carbohydrate content O.D at 630nm	Concentration (µg/ml)
Initial Broth	0.54	54	0.34	34
After inoculation	0.39	39	0.22	22

Estimation of carbohydrate

The carbohydrate content was estimated by Anthrone method[7]. 1ml of culture filtrate was added to 4

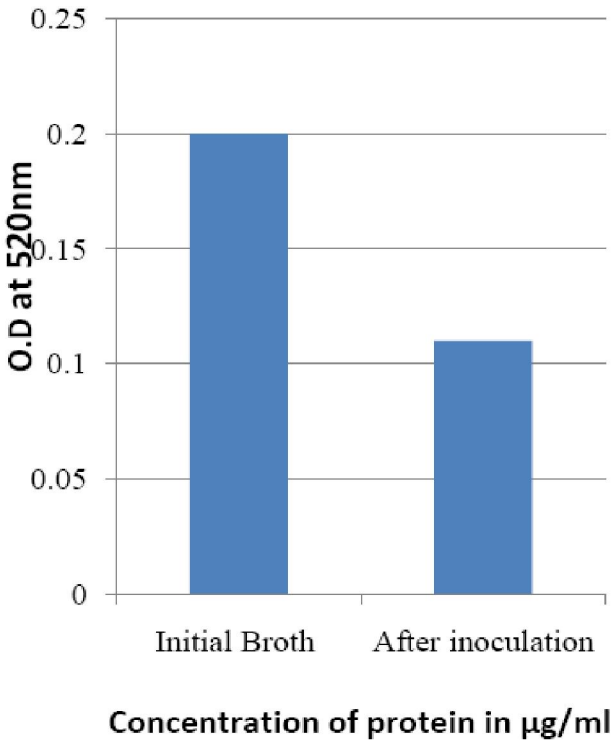

Figure 1 : Protein estimation (bacteria)

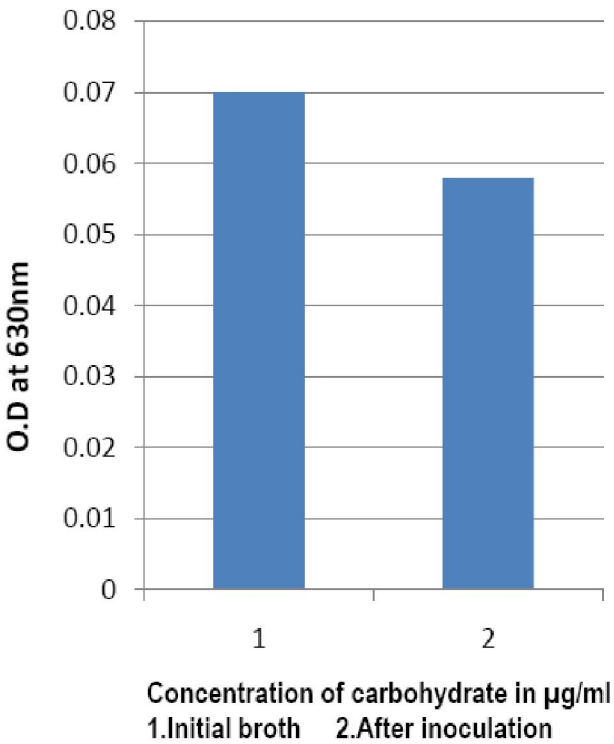

Figure 2 : Carbohydrate estimation (bacteria)

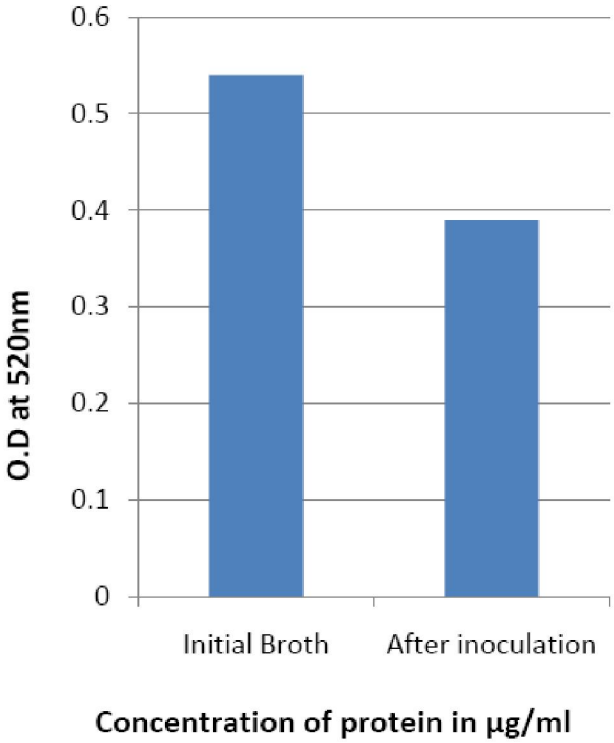

Figure 3 : Protein estimation (fungi)

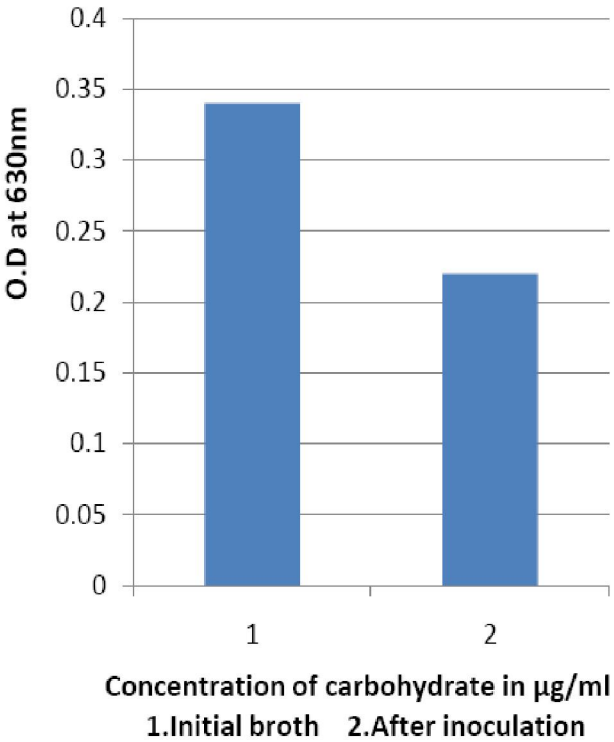

Figure 4 : Carbohydrate estimation (fungi)

ml of Anthrone reagent and heated for 8 minutes in a boiling water bath. Then it was cooled rapidly to room temperature and the absorbance was read at 630 nm. (TABLE 3 and TABLE 4, Figure 3 and Figure 4).

REUTILIZATION OF MEDIA

The culture filtrate was divided into three parts:

FULL PAPER

- First part consisting of 100 ml broth was resupplemented as of original nutrient broth values (i.e., protein and carbohydrate contents) for bacteria and Asthana & Hawker's broth values for fungi.
- Second part consisting of 100 ml broth was not resupplemented.
- Third part consisting of 50 ml broth was resupplemented (as control).

First two parts of the culture filtrate were reinoculated with the same organism as initial and incubated at 37°C for 48 hours for bacteria and 28°C for 5 days for fungi. After the incubation period the biomass was calculated as mentioned earlier. Then the broth was centrifuged at 5000 rpm for 10 minutes. After the centrifugation the supernatant was collected and its protein and carbohydrate contents were estimated by above stated methods (TABLE 5 and TABLE 6, Figure 5, Figure 6, Figure 7 and Figure 8).

TABLE 5 : Protein and carbohydrate content under different conditions (for bacteria)

	Protein content O.D at 520nm	Concentration (µg/ml)	Carbohydrate content O.D at 630nm	Concentration (µg/ml)
100ml broth with supplement	0.16	30	0.04	2
100ml broth with no supplement	0.14	26	0.02	1
50ml broth with supplement (control)	0.20	40	0.1	10

TABLE 6 : Protein and carbohydrate content under different conditions (for fungi)

	Protein content O.D at 520nm	Concentration (µg/ml)	Carbohydrate content O.D at 630nm	Concentration (µg/ml)
100ml broth with supplement	0.05	10	1.0	10
100ml broth with no supplement	0.03	6.5	0.06	6
50ml broth with supplement (control)	0.05	10	0.42	4.2

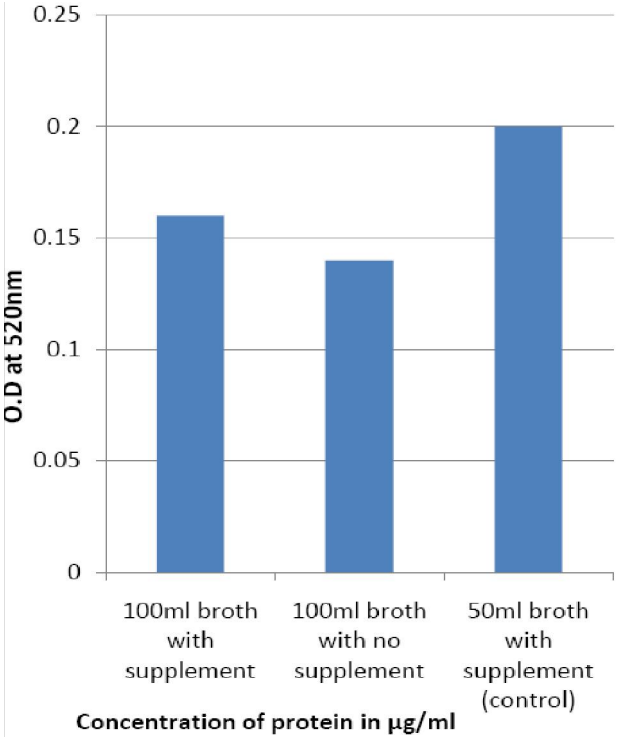

Figure 5 : Protein estimation (bacteria)

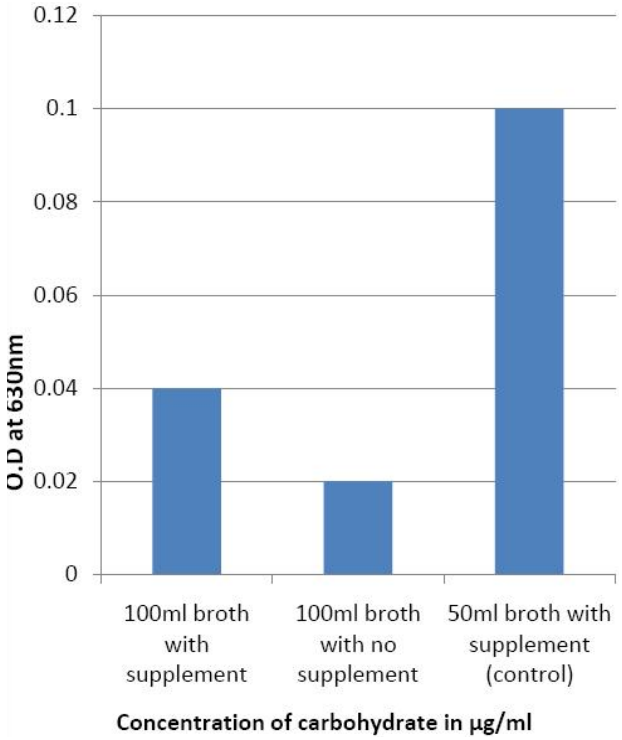

Figure 6 : Carbohydrate estimation (bacteria)

RESULTS AND DISCUSSION

The present investigation revealed that the protein content and carbohydrate content decreased when the medium was reutilized and the growth was also very less when compared to fresh medium. The protein content in initial broth (i.e., before inoculation) for bacteria

FULL PAPER

was 40 µg/ml and for fungi it was 54 µg/ml. After inoculation the protein content was 22 µg/ml (bacteria) and 39 µg/ml (fungi). The carbohydrate content in initial broth for bacteria was 70 µg/ml and 34 µg/ml for fungi. After inoculation the carbohydrate content was 50 µg/ml (bacteria) and 22 µg/ml (fungi). When the media was resupplemented with deficient concentrations of protein and carbohydrate, the protein concentration was 30 µg/ml (bacteria) and 10 µg/ml (fungi) and the carbohydrate concentration was 2 µg/ml (Bacteria) and 10 µg/ml (TABLE 5 and 6, Figures 5 and 6). The media when reutilized without supplementing the lost protein and carbohydrate content the protein concentration was 26 µg/ml for bacteria and for fungi it was 6.5 µg/ml and the carbohydrate concentration was 1 µg/ml (bacteria) and 6 µg/ml for fungi (Figures 7 and 8).

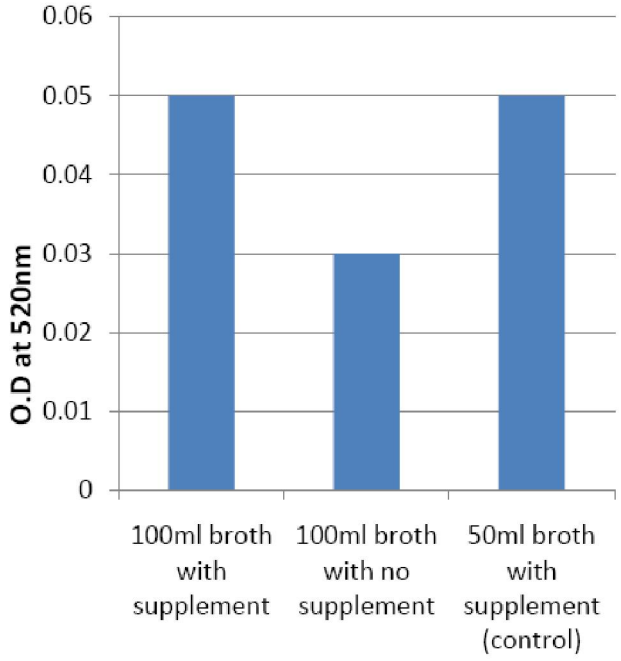

Figure 7 : Protein estimation (fungi)

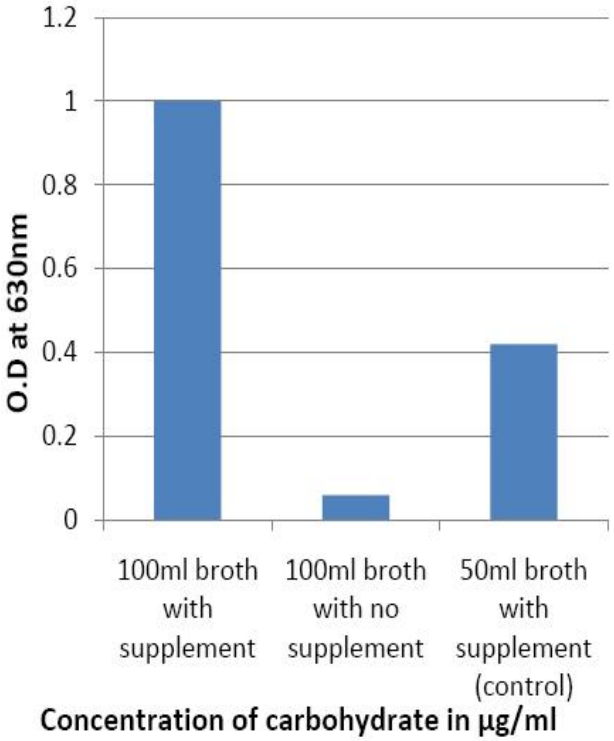

Figure 8 : Carbohydrate estimation (fungi)

The difference in yield may be due to accumulation of secondary metabolites, toxic compounds or low levels of the media constituents which might be responsible for the lower levels of yields. Secondary metabolism is altered by altering growth conditions and media compositions, in case of bacteria and fungi. Secondary metabolites such as alkaloids, latex, and antibiotics are of great economic value for man. Therefore, such compounds can be synthesized in large quantities by manipulating culture conditions, or by adding the necessary precursors in the media of the cultures.

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