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To evaluate Lab scale Cultivation of *Spirulina* by using different substrates and to Evaluate its Chlorophyll and Protein content

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Abstract

Spirulina is a multicellular, filamentous, free floating cyanobacterium or photosynthetic blue green algae. Spirulina has been so popular in the world due to its high nutritional contents. As it contains chlorophyll 'a', like higher plants botanist classify it as micro algae belonging to Cyanophyceae class; but according to bacteriologists it is a bacterium due to its prokaryotic structure. Mexicans started using this microorganism as human food. The nutritional status of cultured Spirulina suggested that the biomass of Spirulina is nutritionally rich in protein. Cyanobacterium Spirulina is proficient to cultivate in a variety of culture media. The present investigation is carried out to evaluate the lab scale cultivation of Spirulina by using different substrates like cheese whey, cow urine, rain water and tube well water. Also it is evaluated for its chlorophyll and protein content. Different concentration gradients of the substrates i.e. cheese whey, cow urine, rain water and tube well water ranges from 10^{-1} to 10^{-6} with ZARROUK'S medium were analyzed for Spirulina growth at pH 9.5 ± 2, temperature 30°C ± 2 and photo-period of 10-12 hours. Spirulina was successfully cultivated by using different substrates and maximum chlorophyll and protein content was founded. The results indicate the potentiality of all the four substrates to provide nutrients to culture medium that reduces its valuable cost and make it a cheaper and economic medium to cultivate Spirulina.

Keywords: *Spirulina*, Biomass, Chlorophyll.

Introduction

Spirulina named as tecuitlatl by Aztecs (Mexicans) that means stone's excrement during 16th century. Later due to outbreak of contagious disease, new customs were adopted by people such as new food, religious and social changes and the topic of tecuitlatl came to an end[1]. *Spirulina*-"small cakes made of mud like algae, which has a cheese-like flavor, and that natives took out of the lake to make bread". They are dried into cakes called "Diha" or"Die". *Spirulina* are classified as *Cyanobacterium* a Gram-negative bacterium which performs photosynthesis same as that occur in plants. *Spirulina* is filamentous, photosynthetic, autotrophic, unbranched and multicellular blue-green algae with symbiotic bacteria that fix atmospheric nitrogen by the process of nitrogen fixation from air. Their main photosynthetic pigment is phycocyanin, which is blue in color and it also contains some other natural pigments such as chlorophyll a, β-carotene, xanthophylls, and allophycocyanin which are responsible for the color of cyanobacterium [2]. *Spirulina* is mainly found in natural lakes having high pH value i.e. 8-10 all over the earth. Because of its alimentary value, *Spirulina* has been consumed from a long past time in many parts of the world as a food supplement by human as well as animals in various forms like health drink, tablets and powder etc.[3]. In Europe, Japan and North America cyanobateris tablet has been sold. *Spirulina* known to be richest source of protein and vitamin and can be used to treat children suffering from malnutrition[4]. The major constituents of *Spirulina* includes protein, vitamin B12, iron, essential amino acids etc., It has been considered as ''Food of the future'' and an ideal food for astronauts by NASA[5]. Cyanobacteria is receiving an increasing interest due to their potential to produce a diverse range of chemicals and biologically active compounds, such as proteins, lipids, vitamins, carotenoid pigments and polysaccharides. For this, *Spirulina* has to be cultivated in commercial way[6].

Material and Methods

Strain procurement: *Spirulina* sp. (NCIM No. 5143) used in the present study was procured from National Collection of Industrial Microorganisms (NCIM) laboratory, Pune- India.

Strain maintenance: For *Spirulina* cultivation, Zarrouk's Medium was used in the present study. Care was taken that the pH of medium is 9.5 after autoclaving. To achieve this, pH of the medium was adjusted to 8.5 which resulted in a pH of 9.5, after autoclaving and "recovery" of the medium. Growth and maintenance of culture is done at 30 ± 2 °C under 12/12 hour light-dark cycles. Culture was stirred manually four times a day after every two hours [7].

Sample Preparation: - For the present study various substrates viz. fresh cheese whey, rain water, tube well water and cow urine were collected from Mohali, Punjab, (India). Serial dilutions were prepared ranging from 10^{-1} to 10^{-6} in distilled water and 10 ml of each dilution were added to 100 ml of sterile

separate zarrouk's medium inoculated with 1ml of *Spirulina* pure culture at pH 9.5 ± 2, temperature 30°C ± 2 and light intensity 2-3 Klux.

Experimental Design: Growth Conditions: For suitable growth, 1 ml of *Spirulina* culture was inoculated in flasks containing 100 ml growth medium and 10 ml dilutions (10^{-1} to 10^{-6}) for each substrate. These flasks were placed under light chamber (3 Klux light intensity) at 30°C to 35°C.

PH Effect: *Spirulina* culture (1ml) was inoculated in flasks (250 ml capacity) containing 100 ml growth medium with range of dilutions for each substrate. pH of the culture used to vary, so it was adjusted daily to range between 8.5-10 pH. Agitation of the culture was done by manual shaking.

Nutrient Requirements: For proper growth of *Spirulina*, the original growth medium (Zarrouk's Media) was modified by adding A_5 micronutrient solution[8].

In the experiment, culture flasks containing 100 ml of Zarrouk's medium and varying dilutions for each substrate were inoculated from stock culture of *Spirulina* that was in exponential phase. Growth rate was measured by taking O.D (optical density) at 560nm. Agitation was done manually, four to five times a day, after regular interval of time. During cultivation, pH was monitored and controlled within values ranging from 8.5 to 10.

The yield of cultures is expressed as total dry weight after 15 days of growth. The total dry weight is determined by harvesting the cells and drying it[8].

Analysis: Growth Rate: Growth was measured at 560nm by UV-Visible Spectrophotometer (Thermostat) after 24 hours till fifteenth day of experiment and a graph of O.D v/s days was plotted.

Harvesting of Cells: Filtration was done by cloth filtration (cotton sari, folded, approximately 20-µm). After filtration, cells were washed with fresh water, dewatered and dried[2].

Total Chlorophyll: Two ml of culture were collected in a centrifuge tube and centrifuged at 2000g for 2 min. The precipitate was suspended in 5 ml methanol for 5 min at 70°C in a water bath, and subsequently centrifuged. Optical density of supernatant was recorded at 655nm by using UV Spectrophotometer[8].

Total Protein: Total protein estimation was done by Lowry's method[9].

Results and Discussion

Spirulina was successfully cultured as liquid media, in a light chamber (3 K-lux light intensity) at 30°C to 35°C temperature In order to compare the growth rate between the substrate used in present study, Zarrouk's medium was formulated (by adding supplements) with addition of ten ml of substrate with concentrations ranging from 10^{-1} to 10^{-6} dilutions in different flasks. One ml of *Spirulina* was inoculated and incubated in flasks containing each substrate dilutions in Zarrouk's medium and O.D was taken at 560 nm, after every 24 hours to check the growth rate After fifteen days of growth cycle dry weight , chlorophyll and protein estimation was done.

Growth performance of *Spirulina* with Cheese whey as a substrate: Comparative growth rate of *Spirulina* was measured in Zarrouk's medium supplemented with cheese whey for 15 days in all dilutions (10^{-1} to 10^{-6}).After 15 days, highest growth was noted in the flask with 10^{-6} dilution, figure 1. The total amount of dry biomass of *Spirulina* in cheese whey substrate was recorded 1.09 g/l. Chlorophyll and protein content of each dilution after 15 days growth cycle of *Spirulina* was measured in Zarrouk's media supplemented with cheese whey, figure 2 and 3.

Growth performance of *Spirulina* with **Cow urine as a substrate:** Comparative growth rate of *Spirulina* was measured in Zarrouk's medium supplemented with cow urine for 15 days in all dilutions (10^{-1} to 10^{-6}). After 15 days, highest growth was noted in the flask with 10^{-6} dilution, figure 4. The total amount of dry biomass of *Spirulina* in cow urine substrate was recorded 1.37 g/l, which was more than cheese whey as supplemented substrate. Chlorophyll and protein content of each dilution after 15 days growth cycle of *Spirulina* was measured in zarrouk's media supplemented with cow urine, figure 5 and 6. After 15 days, highest growth was noted in the flask with 10^{-6} dilution.

Growth performance of *Spirulina* with rain water as a substrate: Comparative growth rate of *Spirulina* was measured in Zarrouk's medium supplemented with rain water for 15 days in all dilutions (10^{-1} to 10^{-6}). After 15 days, highest growth was noted in the flask with 10^{-6} dilution, figure 7. The total amount of dry biomass of *Spirulina* in rain water substrate was recorded 2.3 g/l, which was more than that of cheese whey and cow urine as substrate. Chlorophyll and protein content of each dilution after 15 days growth cycle of *Spirulina* was measured in zarrouk's media supplemented with rain water, figure 8 and 9.After 15 days, highest growth was noted in the flask with 10^{-6} dilution.

Growth performance of *Spirulina* with Tube well water as a substrate: Comparative growth rate of *Spirulina* was measured in Zarrouk's medium supplemented with tube well water for 15 days in all dilutions (10^{-1} to 10^{-6}). After 15 days, highest growth was noted in the flask with 10^{-6} dilution, figure 10. The total amount of dry biomass of *Spirulina* in tube well water substrate was recorded 2.0 g/l which was higher than cheese whey and cow urine. Chlorophyll and protein content of each dilution after 15 days growth cycle of *Spirulina* was measured in zarrouk's media supplemented with tube well water, figure 11 and 12. After 15 days, highest growth was noted in the flask with 10^{-6} dilution.

Comparative statistical analysis (mean) of growth, protein and chlorophyll content with different substrates: Table -1 revealed high growth rate in zarrouk's media supplemented with rain water, followed by Zarrouk's media supplemented with tube well water, Zarrouk's media supplemented with cow urine and lowest in zarrouk's media supplemented with cheese whey. Protein estimation was observed higher on fifteenth day in zarrouk's media supplemented with rain water followed by Zarrouk's media supplemented with cheese whey, Zarrouk's media supplemented with cow urine and lowest in zarrouk's media supplemented with tube well water. whereas in term of chlorophyll content the highest content observed in_zarrouk's media supplemented with tube well water which followed by Zarrouk's media supplemented with cow urine, Zarrouk's media supplemented with rain water and Zarrouk's media supplemented with cheese whey. So this study demonstrated that all these supplements can be used in additional with media to grow *Spirulina* commercially and overall it was observed that rain water as supplement had much potential to grow *Spirulina* of commercial value. Further it was shown by Endrie[10] that the high protein content and rapid growth rate of *Spirulina* was in alkaline medium. He cultivated *Spirulina* by outdoor cultivation technology using a local product (Trona) as a substitute for three major ingredients ($NaHCO_3$, Na_2CO_3 and NaCl) in Zarrouk's standard culture medium. In our study we used different substrates as a substitute in Zarrouk's media for commercial production of *Spirulina*, which can be a good protienaceous source as food supplement. It was observed and founded that by using rain water as a substitute in Zarrouk's media high growth yield and high protein content was seen. Jain and Singh[11] have shown that Cyanobacterium *Spirulina* is capable to grow in various kinds of culture media. They formulated a low cost medium using cow dung ash for the biomass production (1.212 g/L dry biomass) of *Spirulina platensis.* In our study we used cow urine as a substrate and the biomass production (1.37g/L dry biomass) was observed. These results indicate the potentiality of these substrates to provide the nutrients to the culture medium.

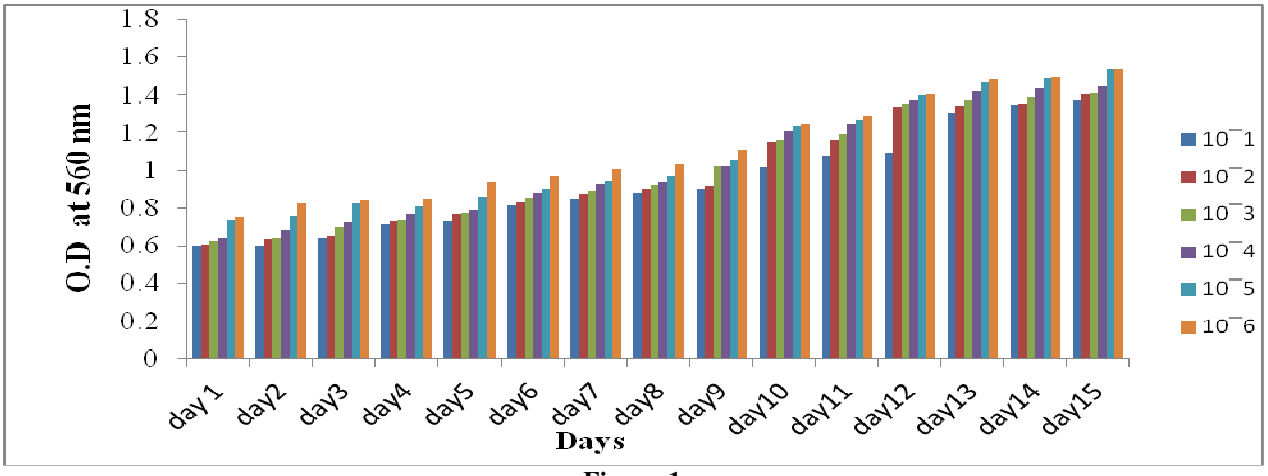

Figure-1
Comparative growth rate of *Spirulina* in Zarrouk's medium supplemented with cheese whey for 15 days in all dilutions (10^{-1} to 10^{-6})

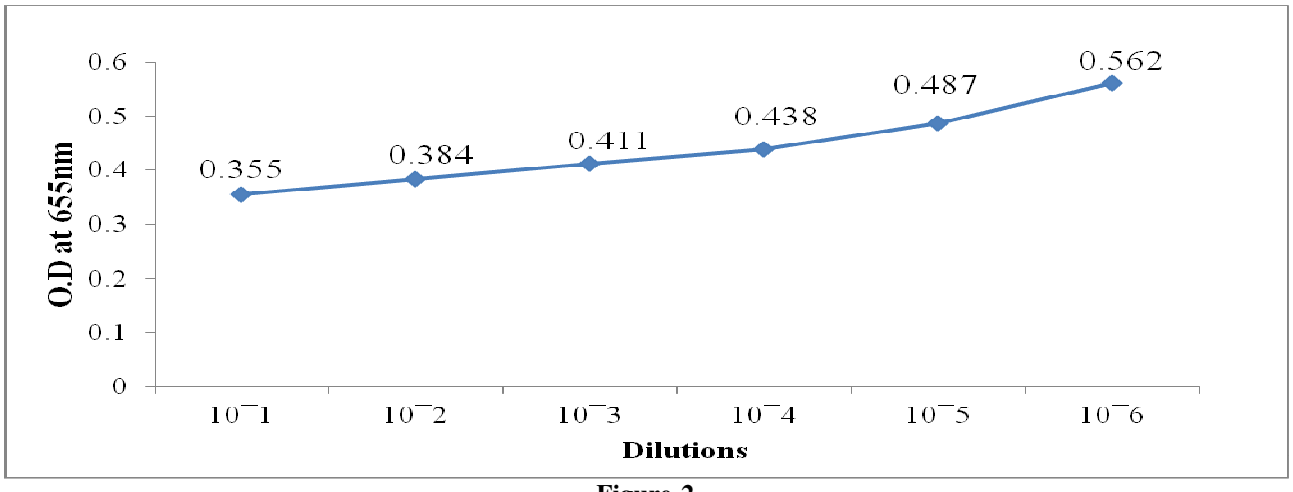

Figure-2
Chlorophyll content of each dilution after 15 days growth cycle of *Spirulina* in Zarrouk's media supplemented with cheese whey

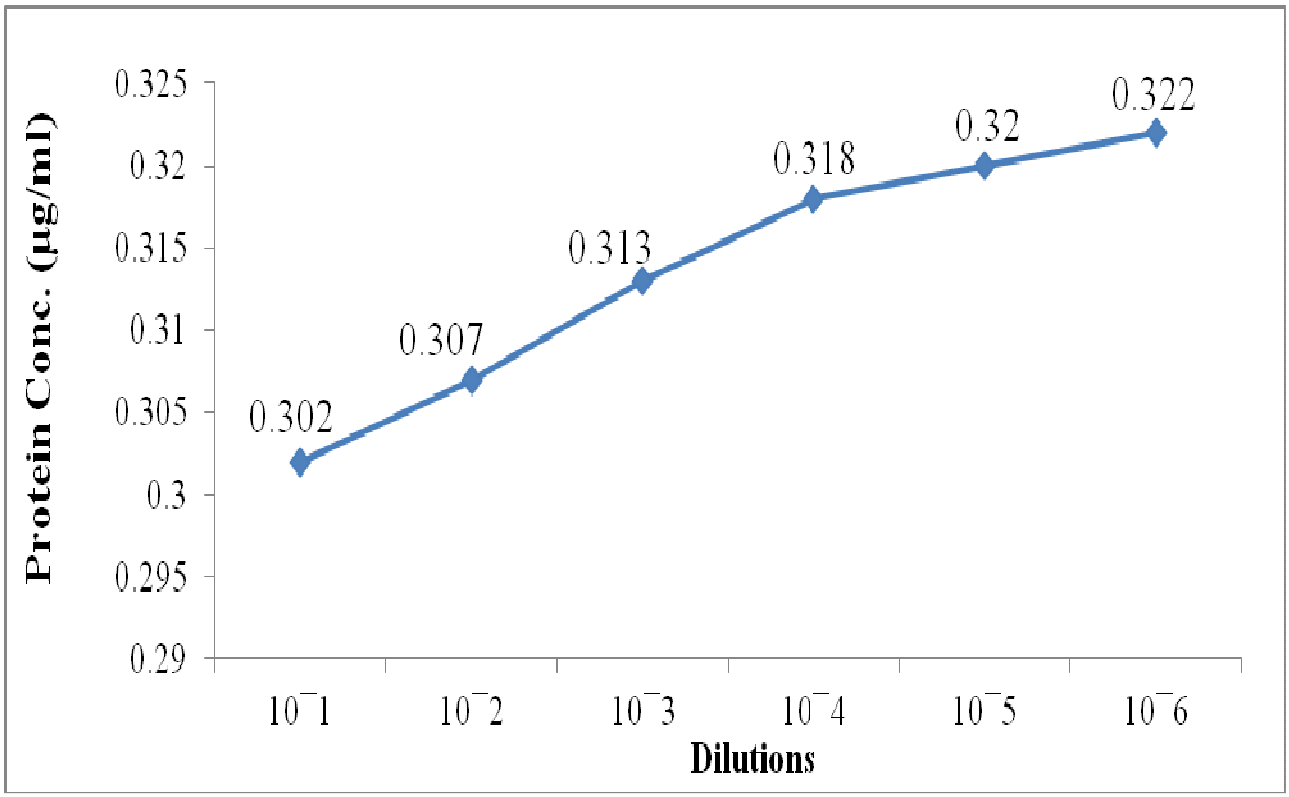

Figure-3
Protein content of each dilution after 15 days growth cycle of *Spirulina* in zarrouk's media supplemented with cheese whey

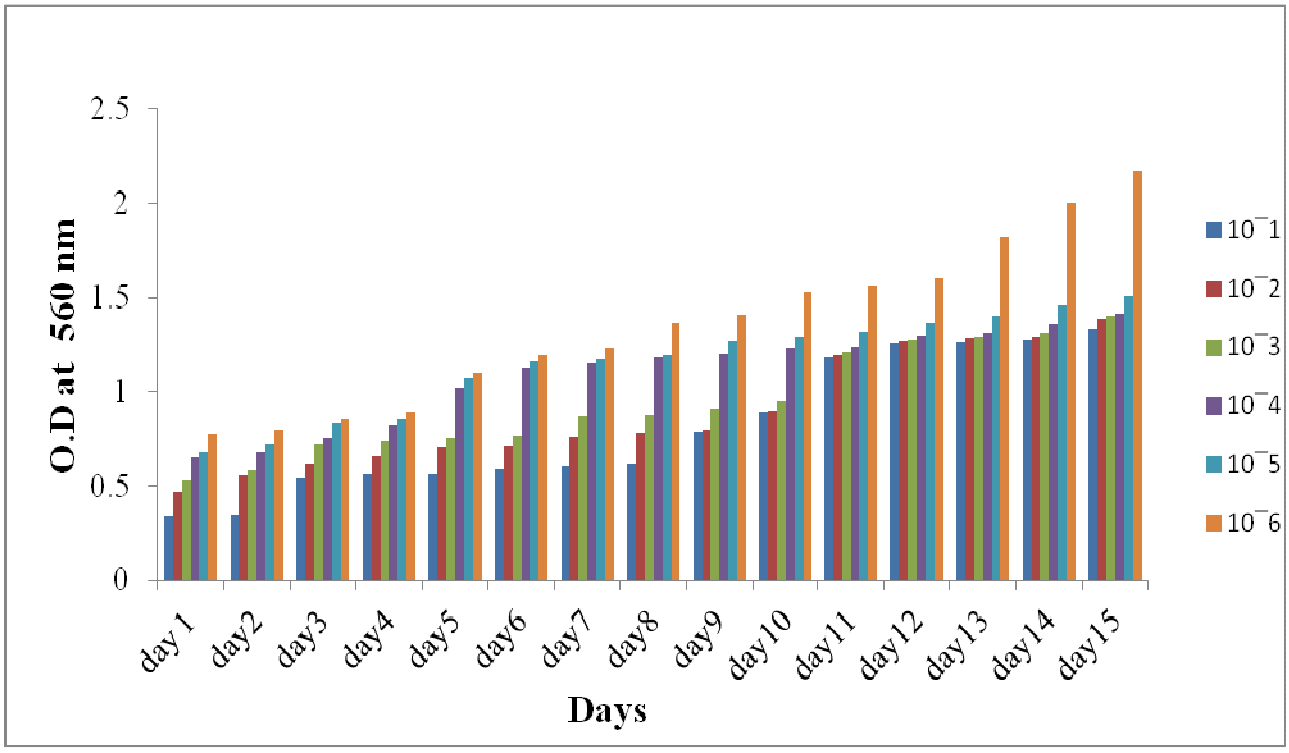

Figure-4
Comparative growth rate of *Spirulina* in Zarrouk's medium supplemented with cow urine for 15 days in all dilutions (10^{-1} to 10^{-6})

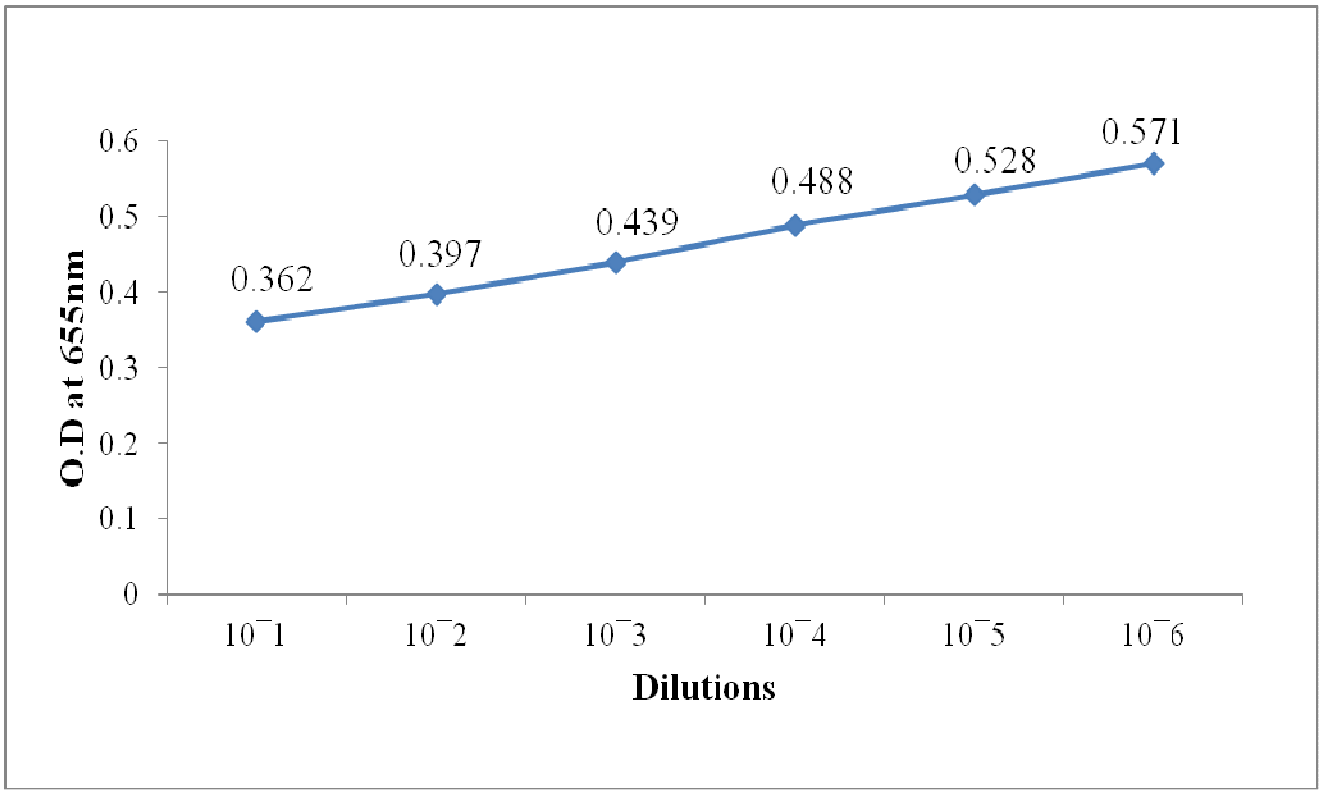

Figure-5
Chlorophyll content of each dilution after 15 days growth cycle of *Spirulina* in Zarrouk's media supplemented with cow urine

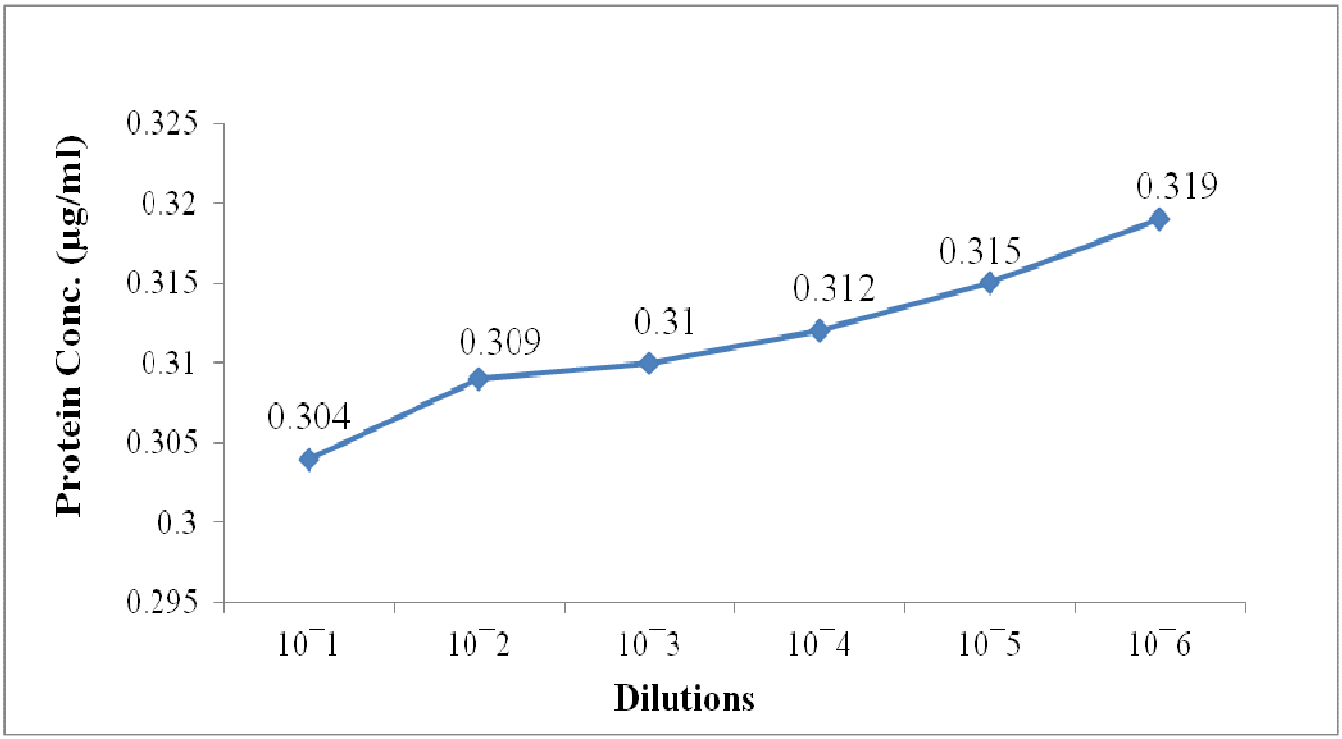

Figure-6
Protein content of each dilution after 15 days growth cycle of *Spirulina* in Zarrouk's media supplemented with cow urine

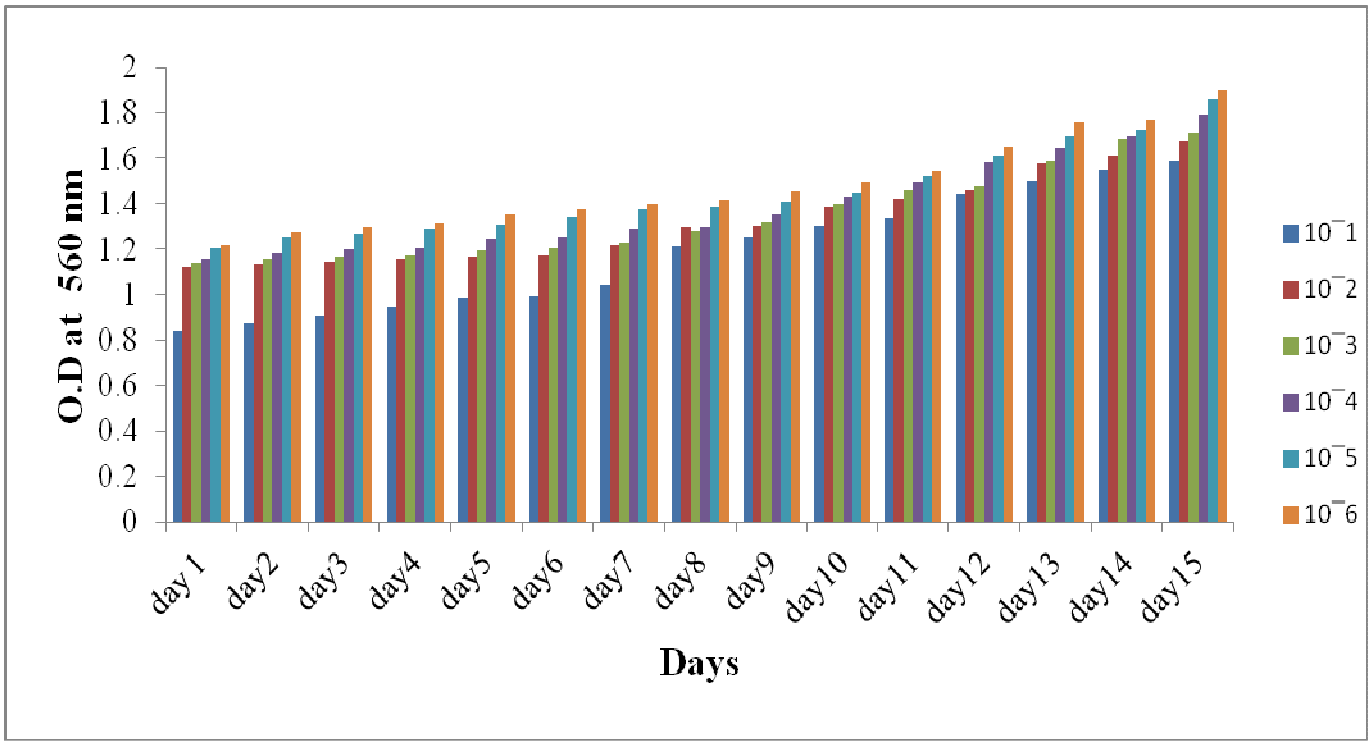

Figure-7
Comparative growth rate of *Spirulina* in Zarrouk's medium supplemented with rain water for 15 days in all dilutions (10^{-1} to 10^{-6})

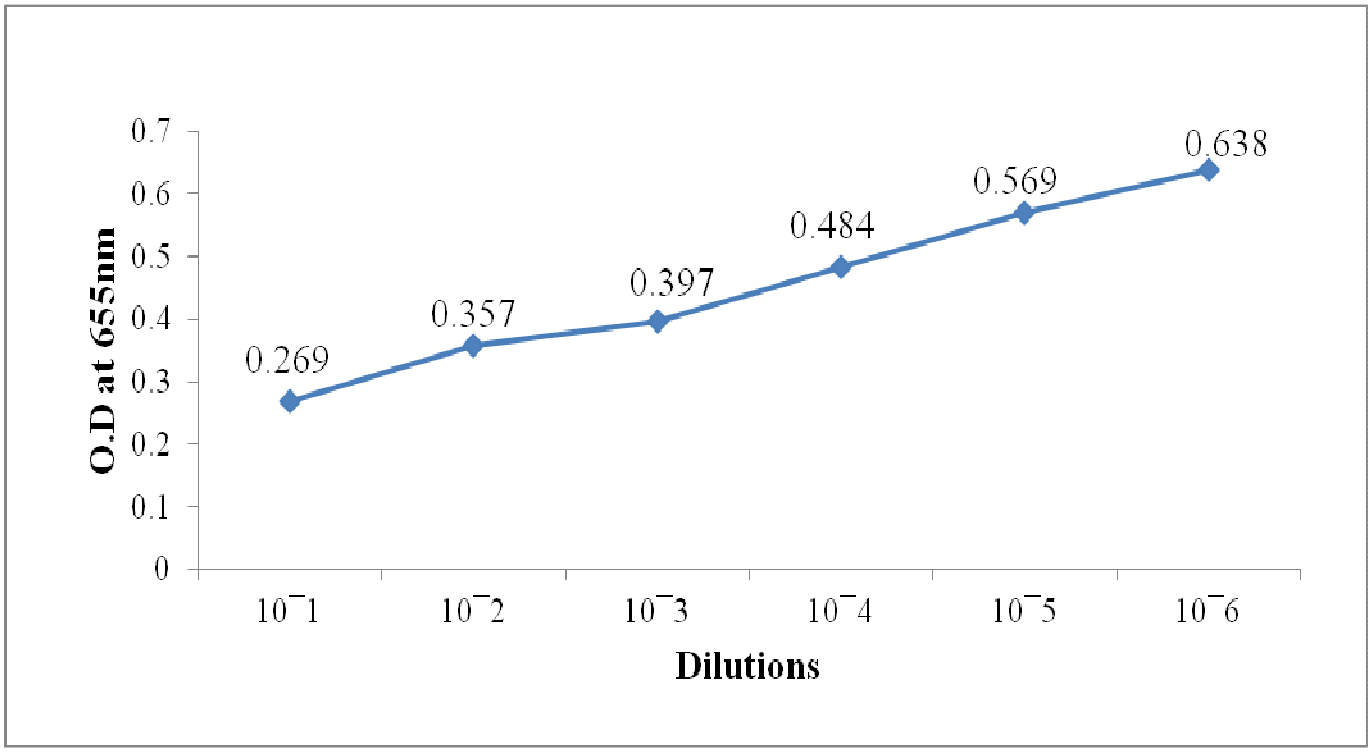

Figure-8
Chlorophyll content of each dilution after 15 days growth cycle of *Spirulina* in Zarrouk's media supplemented with rain water

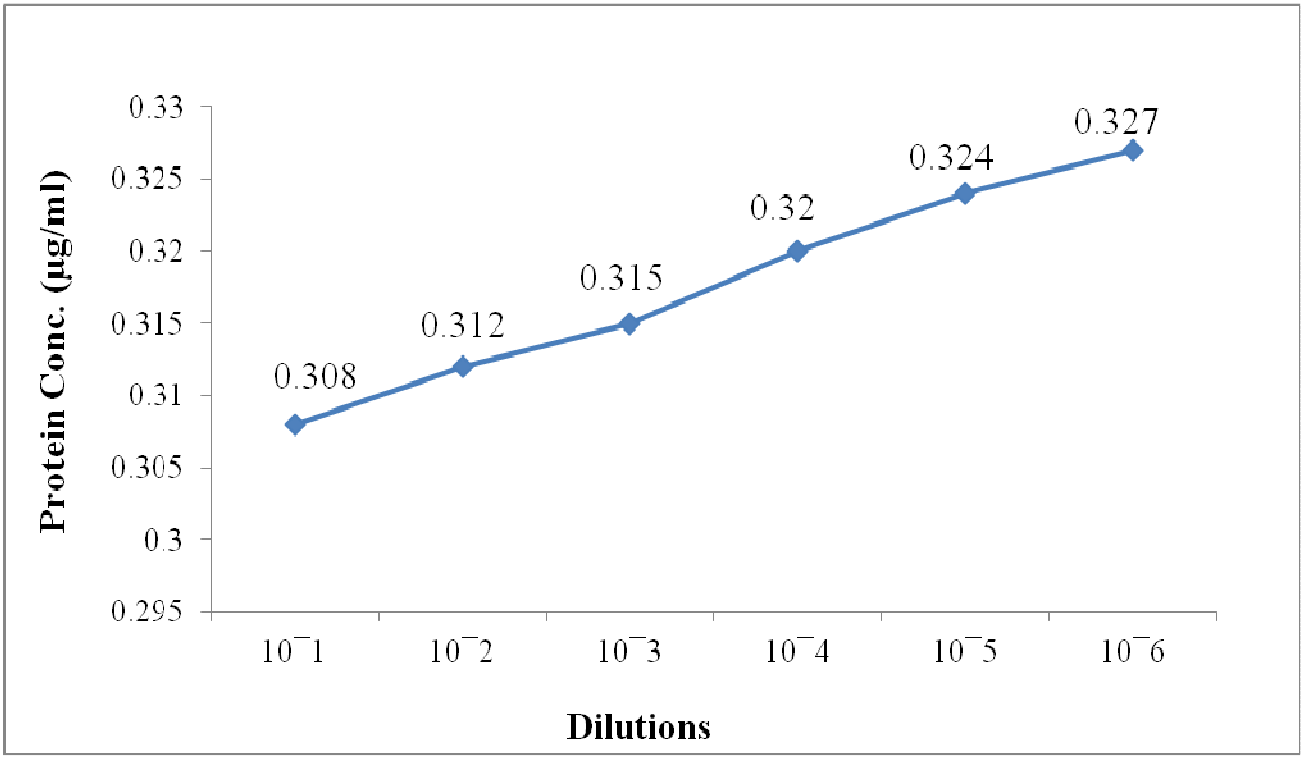

Figure-9
Protein content of each dilution after 15 days growth cycle of *Spirulina* in Zarrouk's media supplemented with rain water

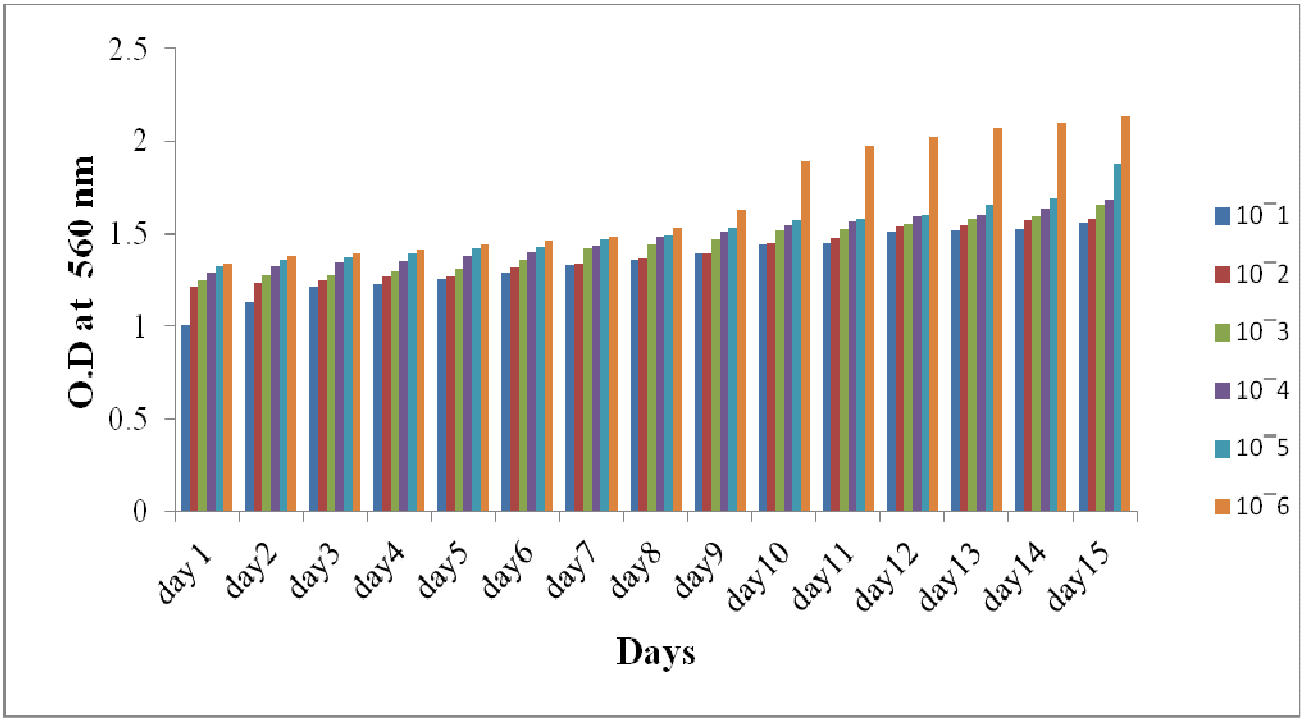

Figure-10
Comparative growth rate of *Spirulina* in Zarrouk's medium supplemented with tube well water for 15 days in all dilutions (10^{-1} to 10^{-6})

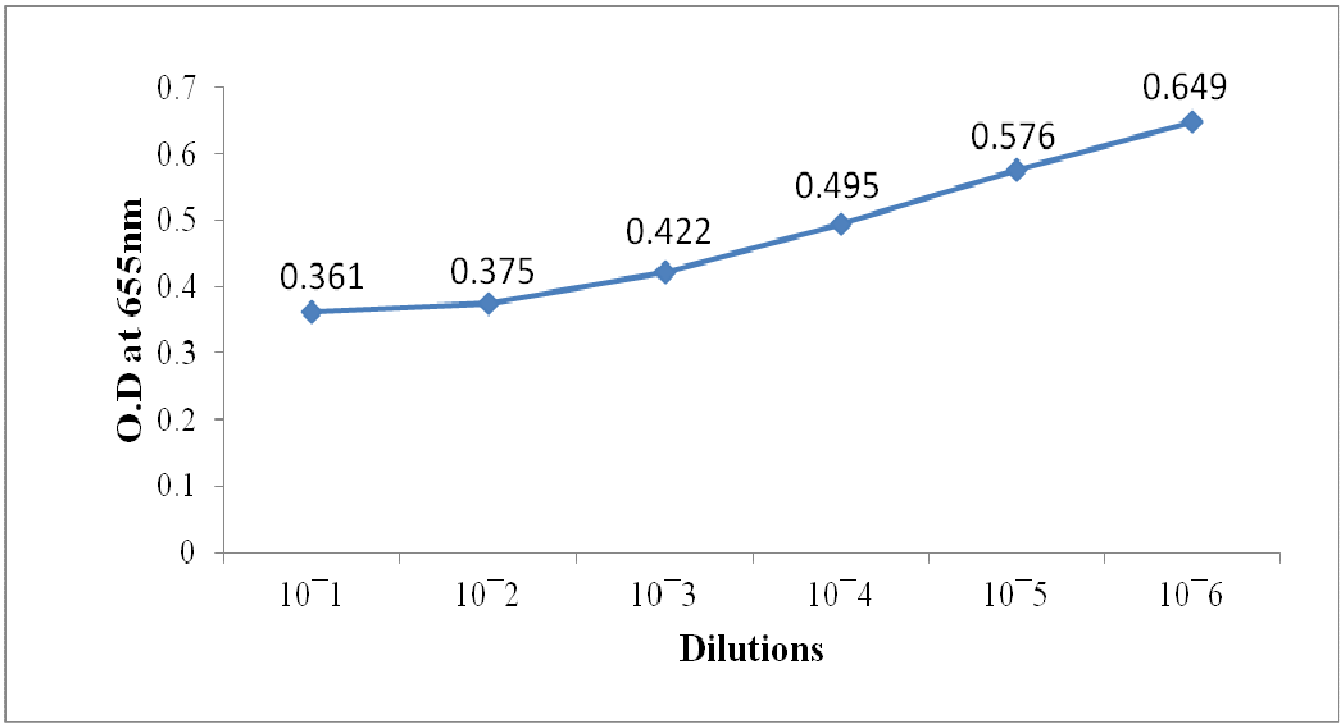

Figure-11
Chlorophyll content of each dilution after 15 days growth cycle of *Spirulina* in Zarrouk's media supplemented with tube well water

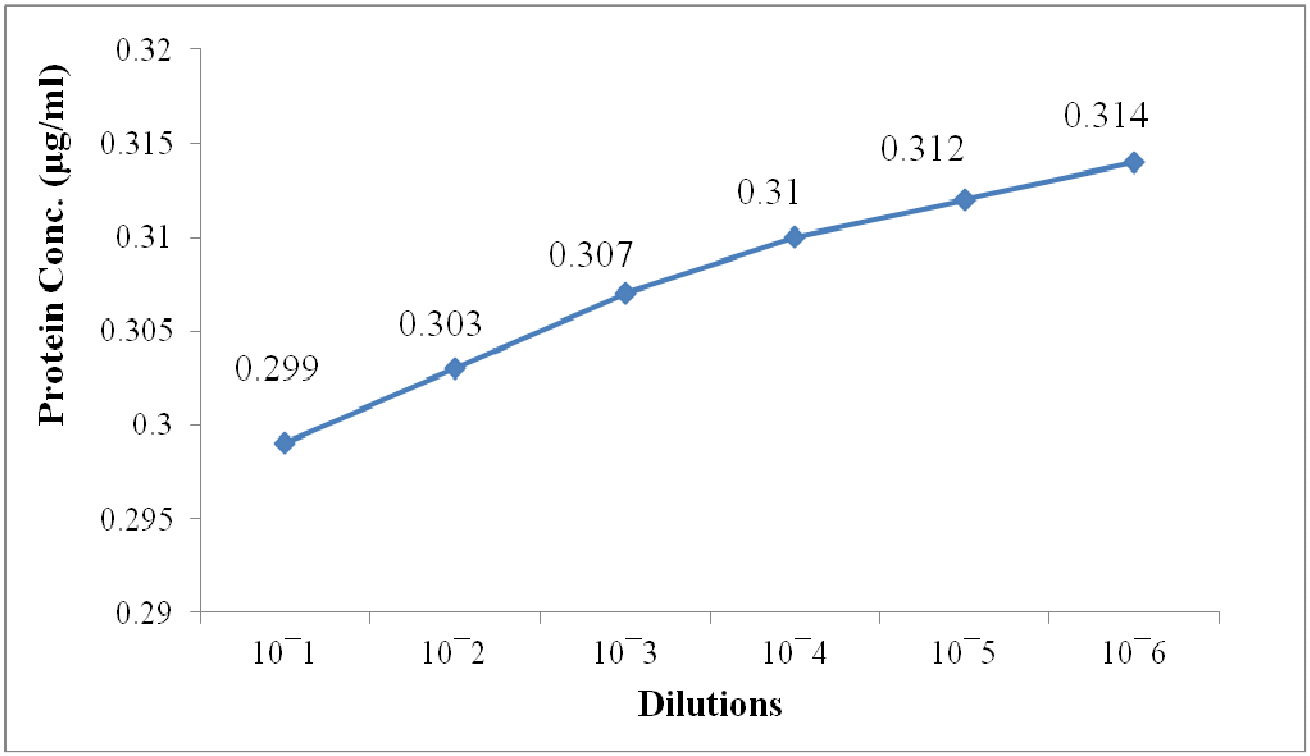

Figure-12
Protein content of each dilution after 15 days growth cycle of *Spirulina* in Zarrouk's media supplemented with tube well water

Table-1
Comparative statistical analysis of Spirulina growth on different supplemented growth mediums

Sr. No.	Growth medium	Average			
		O.D{maximum growth on fifteenth day (O.D at 560nm)}	Protein content{maximum protein content on fifteenth day} (mg/ml)	Chlorophyll content{max chlorophyll content on fifteenth day} (mg/ml)	Dry weight (g/l)
1	Zarrouk's media supplemented with cheese whey	1.45	0.31	0.44	1.09
2	Zarrouk's media supplemented with cow urine	1.53	0.31	0.47	1.37
3	Zarrouk's media supplemented with rain water	1.76	0.32	0.45	2.3
4	Zarrouk's media supplemented with tube well water	1.75	0.31	0.48	2.0

Conclusion

The present study, investigated comparative growth rate of *Spirulina* in Zarrouk's medium supplemented with different substrates. *Spirulina* was cultivated to evaluate lab scale cultivation by using different substrates as growth supplements. *Spirulina* grows well in all the four substrates; with the highest growth performance and protein content when grown in rain water (2.3g/l). The highest chlorophyll content (0.479µg/ml) and protein content (0.317µg/ml) were found in tube well water and rain water respectively.

The experimental data indicates that *Spirulina* when cultivated on varying concentrations of substrate supplementation, yield better growth. It indicates that cheese whey, cow urine, rain water and tube well water has potential to increase the growth rate of *Spirulina. Spirulina* appears to have extensive potential for the growth and development in various types of wastes like cheese whey, cow urine, and rain water.

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