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# Nitrogen fixing ability, protein content and variation in pH values of *Azotobacter* in liquid and carrier based cultures at different intervals

**Abhishek Sharma, RP Ahirwar and NG Mitra**

**Abstract**
The study was performed at Commercial Biofertilizer Production Centre, Deptt. of Soil Science & Agril. Chemistry, JNKVV, Jabalpur comprising total 17 treatment combinations: two different types of (liquid and carrier based cultures of *Azotobacter*) individually maintained at different physical conditions of temperatures viz., 4ºC (refrigeration), 20, 30, and 40 ºC and subjected to different chemical inducers or stressors viz., no chemical, n-butanol, β-hydroxy butyrate and calcium deficiency. Every treatment was replicated thrice under randomized block design. The treatments were tested under both *in vitro* laboratory condition and pot experimentation with chilli crop (cv. Jawahar Mirch 283). Nitrogen fixing ability of liquid and carrier based *Azotobacter* cultures was measure at sixth month growth intervals. The different nitrogen fixing ability of *Azotobacter* cultures ranged from 3.12 to 4.81 mg/unit wt sucrose consumed with the treatment combinations of carrier based culture alongwith Ca-deficiency and liquid based cultures at 30ºC, respectively. The value of 3.79 mg/unit wt sucrose consumed was the average nitrogen fixing ability of *Azotobacter* cultures. The variation in pH values of different cultures at the different intervals ranged from 5.28 to 7.20 with treatment combinations of carrier based culture along with Ca-deficiency and liquid culture maintained at 30ºC, respectively with an overall average pH value 6.21.

**Keywords:** *Azotobacter,* Nitrogen fixing ability *of Azotobacter,* Protein content of *Azotobacter,* bio fertilizer, pH of *Azotobacter* and carrier and liquid cultures

## Introduction
*Azotobacter* is a genus of usually motile, oval or spherical Gram negative bacteria that form thick-walled cysts and may produce large quantities of capsular slime. They are aerobic, free-living soil microbes which play an important role in the nitrogen cycle in nature, binding atmospheric nitrogen asymbiotically, which is inaccessible to plants, and releasing it in the form of ammonium ions into the soil (nitrogen fixation). In addition to being a model organism for studying diazotrophs, it is used by humans for the production of biofertilizers, food additives, and some biopolymers. The first representative of the genus, *Azotobacter chroococcum*, was discovered and described in 1901 by the Dutch microbiologist and botanist Martinus Beijerinck. It also synthesizes some biologically active substances, including some phytohormones such as auxins (Ahmad *et al.*, 2005) [2], there by stimulating plant growth.

The optimal pH for the growth and nitrogen fixation is 7.0–7.5, but growth is sustained in the pH range from 4.8 to 8.5 *Azotobacter* can also grow mixotrophically, in a molecular-nitrogen-free medium containing mannose; this growth mode is hydrogen-dependent. Hydrogen is available in the soil, thus this growth mode may occur in nature. While growing, *Azotobacter* produces flat, slimy, paste-like colonies with a diameter of 5–10 mm, which may form films in liquid nutrient media. The colonies can be dark-brown, green, or other colors, or may be colorless, depending on the species. The growth is favored at a temperature of 20–30 °C.

*Azotobacter* is a genus of usually motile, oval or spherical bacteria producing large quantities of capsular slime and that form thick-walled dormant cysts under adverse conditions. The bacteria proliferate better in soils having pH around neutrality (Gandora *et al.*, 1998) [7]. Indian Standard Specification (1979) has established that the effective bio-inoculants of *Azotobacter* should ensure a viable cell count $10^8$cfu per g of carrier based culture or per ml of liquid culture.

However, the traditional culture of this diazotroph encounters a problem of short shelf-life and lack of survivability of vegetative cells under adverse environmental conditions like temperature and chemicals. On the other hand, cysts of this bacterial genus are more resistant to adverse environmental factors like desiccation, and solar irradiation, also to some extent heating (Socolofsky and Wyss, 1962) [13].

**Material and Methods**

The experiment was conducted in the Centre for Commercial Biofertilizer Production Centre (BPC), Department of Soil Science & Agricultural Chemistry, College of Agriculture, Jawaharlal Nehru KrishiVishwaVidyalaya (JNKVV), Jabalpur (M.P.).

**Types of *Azotobacter* cultures**

**Carrier based culture (CC)**

The conventional *Azotobacter* bioinoculants consisting of vegetative cells of *Azotobacter*in lignite as carrier was used in the experiment. The strain Azo-2 derived from the project on Biological Nitrogen Fixation (ICAR), JNKVV, Jabalpur. The strain was multiplied as required for the present experiment. At all circumstances the microbial population was maintained up to the standard $10^9$cfu/g lignite.

**Liquid based culture (LC)**

In the case of liquid bio-fertilizers, the count of the microbial strain was maintained up to $10^9$cfu/ml broth.

**Inducers for Encystations**

Physical Temperature (for laboratory growth of cultures) at 4 $^oC$ (in refrigerator) $t_0$ and at 20, 30, 40 $^oC$ (in incubator) $t_1$, $t_2$, $t_3$ are respectively. Chemical Inducers n-butanol (0.3%), β-hydroxy butyrate (each 0.3%), and mineral Ca deficiency in culture media $C_1$, $C_2$, $C_3$, and $C_0$ (for no chemical inducer i.e., normal medium)

**Preparation of Bio-cultures**

**Nitrogen fixing organisms (*Azotobacter*)**

**Preparation of carrier material**

The carrier material (lignite) was ground to a fine powder from.The pH of the carrier material was maintained neutral with the help of calcium carbonate (1:10 ratio), since the lignite was acidic in nature (pH of 4-5).The neutralized carrier material was sterilized in an autoclave to eliminate the contaminants. In the mean time, liquid broth culture was obtained from the proved strain following batch culture fermentation. Mixing the carrier and the broth culture was performed following packaging the Inoculants in packets.

**Preparation of inoculants packet**

The neutralized and sterilized carrier material was spread in a clean, dry, sterile metallic or plastic tray. The bacterial broth culture from fermentor was added to the sterilized carrier material and mixed well manually (after wearing sterile gloves) or mechanically in electric mixer. The culture suspension was added to a level of 40-50% of the solid carrier material. The polythene packets each containing bio-culture 200 g were sealed with electric sealer and allowed for curing for 2-3 days at room temperature.

**Preparation of Broth**

Broth was prepared in flasks and inoculum from slant was transferred to flasks. The culture was grown under batch culture fermentation at 30±2°C as submerged culture. The culture was incubated until the desired level of microbial population $10^{10}$ to $10^{11}$ cfu/ml was obtained. Under optimum conditions, this population level could be attained within 6-7 days in case of *Azotobacter*. The culture obtained in the flask is called starter culture. For large scale production of inoculants (mass culture), inoculum from starter culture was transferred to large flasks (cap. 2.5 litre) and grown on horizontal shaker until required level of cell count was attained.

**pH of *Azotobacter* culture**

pH of culture was determined by glass electrode pH meter (Piper, 1950) [12].

**Nitrogen fixing ability of *Azotobacter* culture**

The digestion of samples (individually 1 g of carrier based and 1 ml liquid culture) was carried out separately for determination of nitrogen content using conc. $H_2SO_4$ and digestion mixture of 600 g $K_2SO_4$ + 20 g $CuSO_4$ + 5 g selenium powder. After digestion the volume of digested material was made up to 100 ml with distilled water and stored for chemical analysis.

**Total protein content**

Protein percent in the *Azotobacter* culture was estimated multiplying nitrogen percent of *Azotobacter* culture by factor 6.25.

Protein (%) = Nitrogen (%) x 6.25

**Results and Discussion**

**Nitrogen fixing ability of *Azotobacter* cultures at first month growth interval**

Column 1 in Table 1 depicted data on nitrogen fixing ability of liquid and carrier based *Azotobacter* cultures at first month growth interval. The variation of nitrogen fixing ability of different *Azotobacter* cultures ranged from 8.53 mg/g and 12.89 mg/ml sucrose consumed with the treatment combinations of carrier based culture with Ca-deficiency and liquid culture at 30$^oC$, respectively. The average nitrogen fixing ability of *Azotobacter* cultures was 9.57 mg/unit wt (mg/g and mg/ml for carrier based and liquid formulation, respectively) sucrose consumed.

**Nitrogen fixing ability of *Azotobacter* cultures at second month growth interval**

Column 2 in Table 1 presented data on nitrogen fixing ability of liquid and carrier based *Azotobacter* cultures at second month growth interval. The variation from 8.41 mg/g and 12.79 mg/ml sucrose consumed for nitrogen fixing ability of *Azotobacter* cultures was observed with the treatment combinations of carrier based culture with Ca-deficiency and liquid based cultures treated with 30$^oC$, respectively. The average nitrogen fixing ability of *Azotobacter* cultures was 9.44 mg /unit wt sucrose consumed.

**Nitrogen fixing ability of *Azotobacter* cultures at third month growth interval**

Column 3 in Table 1 exhibited data on nitrogen fixing ability of liquid and carrier based *Azotobacter* cultures at third month growth interval. The variation of nitrogen fixing ability of *Azotobacter* cultures in different cultures ranged from 8.23 mg/g and 12.40 mg/ml sucrose consumed with the treatment combinations carrier based culture with Ca-deficiency and liquid based cultures at 30$^oC$, respectively. The average

nitrogen fixing ability of *Azotobacter* cultures was 9.23 mg /unit wt sucrose consumed.

**Nitrogen fixing ability of *Azotobacter* cultures at fourth month growth interval**

Column 4 in Table 1 presented data on nitrogen fixing ability of liquid and carrier based *Azotobacter* cultures at the fourth month at different growth interval. The variation in nitrogen fixing ability of different cultures ranged from 7.80 mg/g and 12.11 mg /ml sucrose consumed with treatment combinations of carrier based culture with Ca-deficiency and liquid based cultures at 30 ºC, respectively. The average nitrogen fixing ability was 9.02 mg /unit wt sucrose consumed.

**Nitrogen fixing ability of *Azotobacter* cultures at fifth month growth interval**

Column 5 in Table 1 presented data on nitrogen fixing ability of liquid and carrier based *Azotobacter* cultures at fifth month growth interval. The variation of nitrogen fixing ability ranged from 5.07 mg/g and 8.75 mg /ml sucrose consumed with the treatments combinations carrier based culture with Ca-deficiency and liquid based cultures maintained at 30ºC, respectively. The average nitrogen fixing ability of *Azotobacter* cultures was 6.42 mg /unit wt sucrose consumed.

**Nitrogen fixing ability of *Azotobacter* cultures at sixth month growth interval**

Column 6 in Table 1 showed data on nitrogen fixing ability of liquid and carrier based *Azotobacter* cultures at sixth month growth interval. The different nitrogen fixing ability of *Azotobacter* cultures ranged from 3.12 mg/g and 4.81 mg /ml sucrose consumed with the treatment combinations carrier based culture with Ca-deficiency and liquid based cultures at 30 ºC, respectively. The value of 3.79 mg/unit wt sucrose consumed was the average nitrogen fixing ability of *Azotobacter* cultures.

**Average nitrogen fixing ability of *Azotobacter* cultures during entire sixth months growth interval**

Column 7 in Table 1 presented data on nitrogen fixing ability of liquid and carrier based *Azotobacter* cultures during entire six month of growth period. The variation of 6.86 mg /g and 10.63 mg /ml sucrose consumed was the range of nitrogen fixing ability of *Azotobacter* cultures in different cultures with the respective treatments of carrier based culture with Ca-deficiency and liquid based cultures maintained at 30 ºC, respectively. The average nitrogen fixing ability of *Azotobacter* cultures was 7.91 mg/unit wt sucrose consumed.

Concisely, liquid culture maintained that different temperatures at 30, 20, 40 and 4 ºC performed superior result by 54.96%, 42.42%, 37.32% and 30.47%, respectively over that of lowest performing treatment of carrier based culture with Ca-deficiency (6.86 mg/g). Liquid culture when supplemented with no chemical responded in the same range by 52.04% relative to the lowest performing one. This was followed by a group of treatment combinations having carrier based cultures maintained at temperatures of 30 and 20 ºC and carrier based cultures with no chemical supplementation. The performance of remaining treatment combinations were statistically of no significance when compared with that of the lowest one.

Variation on nitrogen fixing ability in liquid and carrier based cultures of *Azotobacter* at different intervals as influenced by chemical and physical inducers/stressors follows the pattern of sixth polynomial equation. The maximum was recorded with liquid culture at 30ºC which was significantly higher relative to other treatments and the minimum value was with the uninoculated control. This was ascertained that the asymbiotic bacteria *Azotobacter* became diazotrophically most active under the optimum conditions of growth with suitable temperature at 30ºC and without any nutritional stress. Conversely, the treatment of untreated or uninocualted control proved itself that the native species of *Azotobacter* were least efficient for diazotrophy. Similar were the results reported by Dalton and Postgate (1969) [5], Abd-el-Malek *et al.* (1979) [1], Inamdar *et al*. (2000) [9] and Joshi *et al*. (2007) [10].

**Protein content in *Azotobacter* cultures at monthly intervals for six month period**

Since protein contents of various liquid and carrier based bioinoculants of *Azotobacter* influenced by different inducers or stressors determined at every month intervals upto 6 months were simply the multiplication values of respective nitrogen contents with a common factor 6.25, therefore, the pattern of data variations (protein contents) were same as those in the nitrogen contents.

However, as interpretation of mean values of protein contents *Azotobacter* culture (average of one to six month intervals), the results varied from 8.44 to 66.41 mg /unit wt with the different treatments combinations, the maximum was with liquid culture of *Azotobacter* maintained at 30ºC which was significantly higher as compared to other treatments. The minimum was recorded with uninoculated control. This drawn an inference that effective diazotrophy contributed better to enhance protein content in the plant system. Similar results were also reported by Stickland (1951) [15], Onwurah (1999) [8], Zubkov *et al*. (1999) [16] and Inamdar *et al*. (2000) [9].

**Variation in pH of *Azotobacter* cultures at first month growth interval**

Column 1 in Table 2 presented data on pH values of liquid and carrier based *Azotobacter* cultures under stresses in first month growth interval. The variation in pH values for different cultures ranged from 5.29 to 7.20 with carrier based culture with Ca-deficiency and liquid culture maintained at 30ºC with average pH 6.36.

**Variation in pH of *Azotobacter* cultures at second month growth interval**

Column 2 in Table 2 depicted data on pH values of liquid and carrier based *Azotobacter* cultures under different conditions at second month growth interval. The variation of pH values in different cultures ranged from 5.40 to 7.25 with the treatment combinations of carrier based culture with Ca-deficiency and liquid culture maintained at 30ºC with an average pH value 6.39.

**Variation in pH of *Azotobacter* cultures at third month growth interval**

Column 3 in Table 2 presented data on variation in pH values for liquid and carrier based *Azotobacter* cultures at third month growth interval. The variation in pH values for different cultures ranged from 5.50 to 7.29 with the treatments carrier based culture with Ca-deficiency and liquid based cultures at 30ºC with average pH values 6.49.

**Variation in pH of *Azotobacter* cultures at fourth month growth interval**

Column 4 in Table 2 presented data on variation of pH values in liquid and carrier based *Azotobacter* cultures at fourth month growth interval. The variation in pH values for different cultures ranged from 5.41 to 7.24 with the treatments of carrier based culture with Ca-deficiency and liquid based cultures treated with 30ºC with an overall average pH values 6.38.

**Variation in pH of *Azotobacter* cultures at fifth month growth interval**

Column 5 in Table 2 expressed data on variation in pH values for liquid and carrier based *Azotobacter* cultures at fifth month growth interval. The variation of pH values in different cultures ranged from 5.31 to 7.23 with the treatment combination of carrier based culture having Ca-deficiency and liquid culture maintained at 30ºC (with average pH values 6.27).

**Variation in pH of *Azotobacter* cultures at sixth month growth interval**

Column 6 in Table 2 presented data on pH values of liquid and carrier based *Azotobacter* cultures at sixth month growth interval. The variation of pH values of in different cultures ranged from 5.28 to 7.20 with treatment combinations of carrier based culture with Ca-deficiency and liquid culture maintained at 30ºC, respectively with an overall average pH value 6.21.

**Average variation in pH of *Azotobacter* cultures during entire sixth months growth interval**

Column 7 in Table 2 depicted data on mean variation in pH values of liquid and carrier based *Azotobacter* cultures during the span of six months growth interval. The variation of pH values in different cultures ranged from 5.37 to 7.24 with treatment combination of carrier based culture with Ca-deficiency and liquid culture maintained at 30ºC (with overall average pH values 6.35).

In overall view, liquid cultures maintained at different temperatures 30, 20, 40 and 4 ºC exhibited superior performance for maintaining pH of media around neutrality with the respective pH values 7.24, 7.05, 6.87, and 6.74. Similarly, liquid culture with no supplementation of chemical and carrier based culture maintained at temperature 30ºC hold the pH at 7.13 and 6.60, respectively. Remaining treatment combinations comprising carrier based and liquid cultures with various other combinations of physical and chemical stresses were unable to maintain media pH values around neutrality.

Variation in pH values in liquid and carrier based cultures of *Azotobacter* at different intervals as influenced by chemical and physical inducers/stressors follows the pattern of sixth polynomial equation. The maximum pH value 7.24 was observed with liquid culture of *Azotobacter* at 30ºC which was significantly higher as compared to other treatment combinations and the minimum value with uninoculated control. This could be attributed to the fact that the optimum temperature 30 ºC required for proliferation of the bacteria would also maintain pH from changing towards acidic. Similar results were also reported by Paczkowski and Berryhill (1979) [11], Chen *et al*. (1985) [4], Frank *et al*. (1989) [6], Boiardi (1994) [3], Inamdar *et al*. (2000) [9] and Stella and Sivasakthivelan (2009) [14].

**Conclusions**

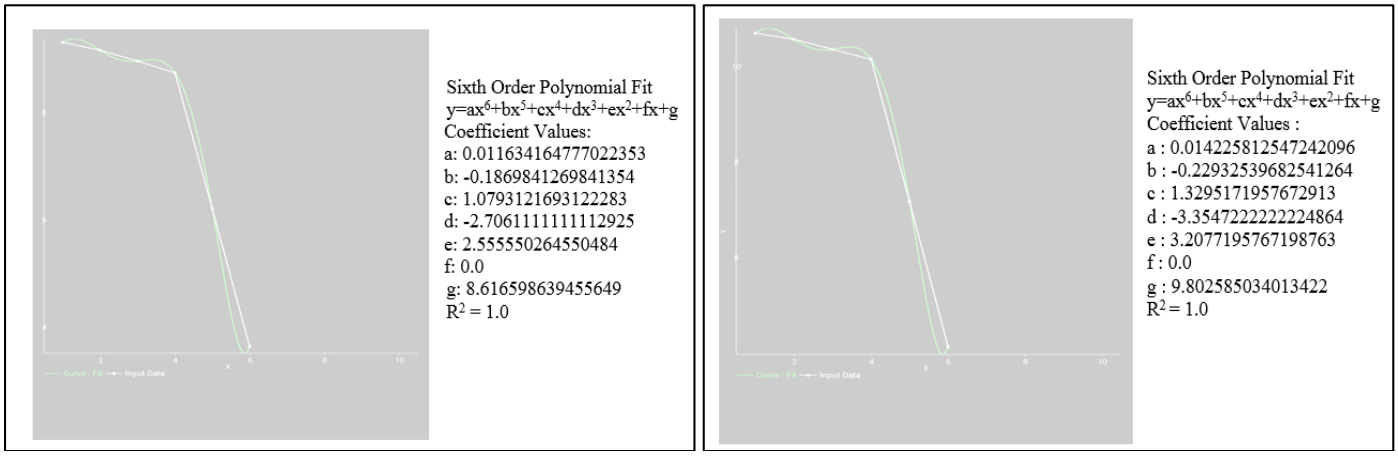
Among different treatment combinations liquid culture of *Azotobacter* maintained at 30ºC responded bearing maximum nitrogen fixing ability and thus protein content by 54.96 and 54.87%, respectively; and resisting pH around neutrality.

**Table 1:** Nitrogen fixing ability and protein content of *Azotobacter* in liquid (mg/ml) and carrier based (mg/g) cultures at different intervals.

| Treatments | 1st month | 2nd month | 3rd month | 4th month | 5thmonth | 6th month | Mean |
|---|---|---|---|---|---|---|---|
| CC + $t_0$ | 9.31 (58.19) | 9.12 (57.00) | 9.01 (56.31) | 8.89 (55.56) | 6.37 (39.81) | 3.77 (23.56) | 7.75 (48.41) |
| CC + $t_1$ | 9.83 (61.44) | 9.69 (60.56) | 9.39 (58.69) | 9.29 (58.06) | 6.89 (43.06) | 3.97 (24.81) | 8.18 (51.10) |
| CC + $t_2$ | 10.35 (64.69) | 10.13 (63.31) | 10.02 (62.63) | 9.83 (61.44) | 7.45 (46.56) | 4.23 (26.42) | 8.67 (54.17) |
| CC + $t_3$ | 9.66 (60.38) | 9.41 (58.81) | 9.25 (57.81) | 9.03 (56.44) | 6.75 (42.19) | 3.89 (24.31) | 8.00 (49.99) |
| CC + $c_0$ | 10.03 (62.69) | 9.92 (62.00) | 9.53 (59.56) | 9.42 (58.88) | 7.18 (44.88) | 4.15 (25.94) | 8.37 (52.32) |
| CC + $c_1$ | 8.64 (54.00) | 8.56 (53.50) | 8.40 (52.50) | 8.18 (51.13) | 5.25 (32.81) | 3.29 (20.56) | 7.05 (44.08) |
| CC + $c_2$ | 8.58 (53.63) | 8.52 (53.25) | 8.33 (52.06) | 7.93 (49.56) | 5.19 (32.44) | 3.20 (20.00) | 6.96 (43.49) |
| CC + $c_3$ | 8.53 (53.31) | 8.41 (52.56) | 8.23 (51.44) | 7.80 (48.75) | 5.07 (31.69) | 3.12 (19.50) | 6.86 (42.88) |
| Mean | 9.37 (58.56) | 9.22 (57.62) | 9.02 (56.38) | 8.80 (54.98) | 6.27 (39.18) | 3.70 (23.14) | 7.73 (48.31) |
| LC + $t_0$ | 10.90 (68.13) | 10.65 (66.56) | 10.27 (64.19) | 10.02 (62.63) | 7.53 (47.06) | 4.35 (27.19) | 8.95 (55.96) |
| LC + $t_1$ | 11.75 (73.44) | 11.69 (73.06) | 11.43 (71.44) | 11.18 (69.88) | 7.98 (49.88) | 4.58 (28.63) | 9.77 (61.06) |
| LC + $t_2$ | 12.89 (80.56) | 12.79 (79.94) | 12.40 (77.50) | 12.11 (75.69) | 8.75 (54.69) | 4.81 (30.06) | 10.63 (66.41) |
| LC + $t_3$ | 11.25 (70.31) | 11.20 (70.00) | 11.03 (68.94) | 10.75 (67.19) | 7.79 (48.69) | 4.49 (28.08) | 9.42 (58.87) |
| LC + $c_0$ | 12.78 (79.88) | 12.43 (77.69) | 12.21 (76.31) | 12.02 (75.15) | 8.45 (52.81) | 4.67 (29.19) | 10.43 (65.17) |
| LC + $c_1$ | 9.01 (56.31) | 8.93 (55.81) | 8.85 (55.31) | 8.73 (54.56) | 6.15 (38.44) | 3.66 (22.88) | 7.56 (47.22) |
| LC + $c_2$ | 8.85 (55.31) | 8.79 (54.94) | 8.71 (54.44) | 8.54 (53.38) | 5.79 (36.19) | 3.58 (22.38) | 7.38 (46.10) |
| LC + $c_3$ | 8.73 (54.56) | 8.63 (53.94) | 8.53 (53.31) | 8.39 (52.44) | 5.45 (34.06) | 3.48 (21.75) | 7.20 (45.01) |
| Mean | 10.77 (67.31) | 10.64 (66.49) | 10.43 (65.18) | 10.22 (63.87) | 7.24 (45.23) | 4.20 (26.27) | 8.92 (55.72) |
| Control | 1.65 (10.31) | 1.54 (9.63) | 1.36 (8.50) | 1.23 (7.69) | 1.17 (7.31) | 1.12 (7.00) | 1.35 (8.44) |
| Grand mean | 9.57 (59.83) | 9.44 (58.97) | 9.23 (57.70) | 9.02 (56.38) | 6.42 (40.15) | 3.79 (23.66) | 7.91 (49.45) |
| $SE_m\pm$ | 0.33 (2.14) | 0.34 (2.36) | 0.34 (2.06) | 0.30 (2.07) | 0.25 (1.51) | 0.14 (0.92) | 0.28 (1.84) |
| $CD_{5\%}$ | 0.96 (6.18) | 0.98 (6.80) | 0.97 (5.95) | 0.87 (5.97) | 0.72 (4.35) | 0.40 (2.66) | 0.82 (5.32) |
| CV (%) | 6.00 (6.20) | 6.24 (6.93) | 6.31 (6.19) | 5.80 (6.35) | 6.68 (6.46) | 6.32 (6.66) | 6.22 (6.46) |

**Table 2:** Variation in pH values in liquid and carrier based cultures of *Azotobacter* at different intervals.

| Treatments | 1st month | 2nd month | 3rd month | 4th month | 5th month | 6th month | Mean |
|---|---|---|---|---|---|---|---|
| CC + $t_0$ | 6.05 | 6.17 | 6.41 | 6.20 | 6.01 | 5.88 | 6.12 |
| CC + $t_1$ | 6.35 | 6.43 | 6.51 | 6.41 | 6.33 | 6.19 | 6.37 |
| CC + $t_2$ | 6.57 | 6.67 | 6.78 | 6.64 | 6.53 | 6.43 | 6.60 |
| CC + $t_3$ | 6.20 | 6.30 | 6.41 | 6.26 | 6.20 | 6.06 | 6.24 |
| CC + $c_0$ | 6.45 | 6.54 | 6.65 | 6.52 | 6.40 | 6.33 | 6.48 |
| CC + $c_1$ | 5.55 | 5.64 | 5.73 | 5.61 | 5.51 | 5.47 | 5.59 |
| CC + $c_2$ | 5.45 | 5.53 | 5.62 | 5.50 | 5.40 | 5.37 | 5.48 |
| CC + $c_3$ | 5.29 | 5.40 | 5.50 | 5.41 | 5.31 | 5.28 | 5.37 |
| Mean | 5.99 | 6.09 | 6.16 | 6.19 | 5.96 | 5.87 | 6.03 |
| LC + $t_0$ | 6.70 | 6.80 | 6.93 | 6.76 | 6.68 | 6.55 | 6.74 |
| LC + $t_1$ | 6.98 | 7.10 | 7.16 | 7.10 | 7.02 | 6.95 | 7.05 |
| LC + $t_2$ | 7.20 | 7.25 | 7.29 | 7.24 | 7.23 | 7.20 | 7.24 |
| LC + $t_3$ | 6.83 | 6.95 | 7.08 | 6.93 | 6.78 | 6.65 | 6.87 |
| LC + $c_0$ | 7.10 | 7.18 | 7.22 | 7.15 | 7.08 | 7.05 | 7.13 |
| LC + $c_1$ | 5.90 | 6.03 | 6.14 | 6.08 | 5.83 | 5.80 | 5.96 |
| LC + $c_2$ | 5.79 | 5.88 | 6.01 | 5.82 | 5.73 | 5.70 | 5.82 |
| LC + $c_3$ | 5.67 | 5.77 | 5.86 | 5.73 | 5.63 | 5.60 | 5.71 |
| Mean | 6.52 | 6.62 | 6.71 | 6.61 | 6.50 | 6.44 | 6.56 |
| Control | 7.01 | 7.03 | 7.04 | 7.02 | 7.00 | 6.98 | 7.01 |
| Grand mean | 6.36 | 6.39 | 6.49 | 6.38 | 6.27 | 6.21 | 6.35 |
| $SE_m\pm$ | 0.24 | 0.26 | 0.25 | 0.24 | 0.23 | 0.21 | 0.24 |
| $CD_{5\%}$ | 0.70 | 0.74 | 0.72 | 0.69 | 0.67 | 0.60 | 0.69 |
| CV (%) | 7.95 | 7.06 | 6.74 | 6.76 | 6.54 | 5.90 | 6.63 |




**Carrier based cultures** **Liquid based cultures**

**Fig 1:** Nitrogen fixing ability in liquid and carrier based cultures of *Azotobacter* at different intervals as influenced by chemical and physical inducers/stressor

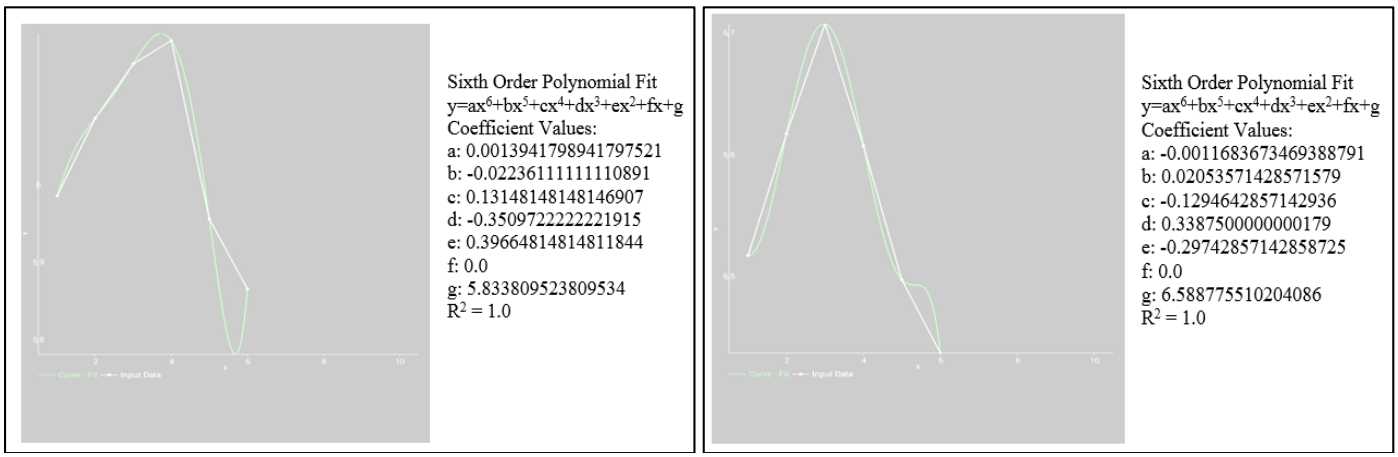



**Fig 2:** Variation in pH values in liquid and carrier based cultures of *Azotobacter* at different intervals as influenced by chemical and physical inducers/stressors

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