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MODIFICATION OF THE PH MEDIUM FOR THE GROWTH OF *AZOTOBACTER*

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Abstract : *Azotobacter, a heterotroph, aerobic organism capable of fixing dinitrogen as non-symbiont. It fixes Nitrogen, produces growth promoting substances and has shown to be antagonistic to pathogens. It increases in growth and yield of crops (Vancura and Macura, 1961). The lack of organic matter in soil is a limiting factor in the proliferation of Azotobacter in soil. The beneficial effects of small amounts of humus on the growth of Azotobacter and its nitrogen fixation are known to us through the work of Jensen (1951), Many workers believe that increased respiration by Azotobacter excludes oxygen from nitrogenase which may serve as natural tool to scavenge oxygen from the site of nitrogen fixation (Dalton and Postgate, 1969 and Postgate, 1971, 1974). It is necessary to find out the growth of bacteria will enhanced so that their ability to survive and synthesis in the surrounding is better.*

In this study considered the hydrogen ion concentration of the Jensen's medium for the better growth of Azotobacter. In first set of the experiment growth was observed 6.6 to 7.6. But in final experiment the pH 6.9 showed better growth of Azotobacter.

Introduction :

Enhancement of growth of microbes depends on the elements and salts used in experimental design made for the type of work and accordinglymedia have been modified by several workers. ACM medium (Shehata and Whitton, 1982) is a modified medium of Kratz and Myers (1955). The optimum growth pH is the most favorable pH for the growth of an organism. The lowest pH value that an organism can tolerate is called the minimum growth pH and the highest pH is the maximum growth pH. The pH of maximum growth rate is called the optimal growth pH. Based on optimal growth pH, microbes can be separated into three groups such as acidophiles grow slow (below pH < 5), neutrophiles grow optimum (pH between 5 and 9), and alkaliphiles grow fast (above pH 9) (Horikoshi, 1999 and Baker-Austin and Dopson, 2007).

Becking (1959) modified Beijerinckia medium for *Azotobacter*. While Sundara Rao and Sinha (1963) modified Pikovskaya's medium for the growth of phosphate solubilizing bacteria. All forms of life, from microorganisms to human beings, share certain nutritional requirements for growth, normal functioning and a source of electrons for their metabolism. Some can use reduced inorganic compounds as electrons donors and termed Lithotrophs (Chemolithotrophs and Photoiithotrophs) other use organic compounds as electrons donors called organotrophs (Chemoorganotrophs and Photoorganotrophs). Nitrogen in some form becomes a cell component. Bacteria are extremely versatile in this respect.

Unlike Eukaryotes, some bacteria can use atmospheric nitrogen and others thrive on inorganic nitrogen compounds as nitrates, nitrites or ammonium and on others organic compounds as amino acids (Yates, 1977). Oxygen is provided in various forms such as water and component atoms of various nutrients, Sulphur is needed for synthesis of certain amino acids (Fridovinch, 1978). Not all biological functions of metal ions are known, but Fe^{z+}, Mg^{t+}, Znt $^{+}$, Mo^{6+}, Mn^{t+} and Cu^{t+} are known to be cof actors for various enzymes. Most bacteria do not require Na^{2+} but certain marine bacteria, cyanobacteria and photosynthetic bacteria do require it. Interpretation of nutritional experiment can depend mainly on the nature of the experimental media and requirements for pH, calcium, magnesium, zinc, copper, molybdenum, boron and chloride in defined media. The plant is not known to need cobalt unless dependent on O_2 (Killian and Father, 1939). Evans and Sorger (1966) reviewed the physiology and biochemistry of these nutrients.

Environmental factors are physical and chemical provide all the raw material for the structural and protoplasmic synthesis of the organisms. Many special proposed media are needed to facilitate recognition, enumeration and isolation of certain types of bacteria. These media provide nutrients that enhance the growth and predominance of a particular type of bacterium and do not enhance other types of organisms that may be present. Cellulose is only carbon source if in a medium will specifically select for or enrich the growth of cellulose-utilizing organisms when it is inoculated with a soil sample containing many kinds of bacteria (Date and Vincent, 1962).

MATERIALS AND METHODS :

The present work was undertaken to modify the media of Jensen's (1942) and for *Azotobacter*. The growth of bacteria was determined by culturing microorganisms in *Azotobacter chroococcum* in Jensen's medium.

Procedure

Prepare 10 ml test tubes containing respective medium. Adjust the pH of each test tube with the help of pH meter using buffer solutions. Autoclave the test tube separately at 15 atm. Pressure for 60 minutes. Take inoculums from 4 days old culture of 0.01 ml of bacteria. Inoculation was added aseptically into sterilized chamber. The growth was estimated in terms of optical density using UV spectrophotometer at 660 nm. This experiment was continued until to make sure that the life cycle of *Azotobacter chroococcum.*

Hydrogen ion concentration (pH)

The hydrogen ion concentration of medium was different with different media. The experiment was set up in the following manner.

(I) in the first set of experiment the range of pH of *Azotobacter* was from 5 to 8.

(II) From the above experimental results, another experiment was set up to find an exact pH needed for more growth of selected organism in the basal medium. The range of pH was 6 to 7 for *Azotobacter* was taken for the study.

RESULTS AND OBSERVATIONS :

Hydrogen ion concentration (pH)

Azotobacter can grow very well near neutral pH. In first set of experiment the *Azotobacter* showed maximum growth in range of 6.6 to 7.4 pH. *Azotobacter* accounted maximum growth at pH 6.9

CONCLUSION AND DISCUSSION:

Hydrogen ion concentration (pH)

The growth and reproduction of the microorganisms are influenced by the pH of the growth medium. Most bacteria show optimal growth inbetween pH 6.5 and 8.5 (Thimann, 1964). *Azotobacter* shows good growth at 6.9. *Azotobacter* is growing luxuriantly in slightly acidic pH.

A fall in pH during the growth of phosphate solubilizing microorganisms has been reported in liquid medium (Sperber, 1957 and Gaur, 1983, 1985). A rise in pH above neutral has also been observed in certain cases (Chhonkar and Subba Rao, 1967). The optimum pH for maximum solubilization of inorganic phosphate has been found to be neutral phosphate to slightly acidic in liquid medium for bacteria and pH 4.0- 5.0 for fungi such as *Penicillum* sp. and *Aspergillus* sp. (Mandel and Resse, 1957). Kolchyns'ka et al (1969) recorded the phosphate activity of *Bacillus substilis* and *B. mesentericus* marked within pH range 4.4 to 10.0 and pH 9.0 proved to be optimum. Whereas, Barskii et al (1989) noted that NADH in respiration of *Bacillus substilis* inhibited at pH 7. The optimum growth pH of *Salmonella* spp. is 7.0–7.5, but the minimum growth pH is closer to 4.2(Nina Parker et al,2011). Syntrophic oxidation, iron reduction, sulphate reduction, and methanol genesis, common microbial redox reactions in anoxic environments (Lovley and Chapelle, 1995 and Bethke et al., 2011). Jin Qusheng and Matthew (2018) showed that energy yields respond strongly to pH variation, which may modulate microbial interactions and help give rise to the pH limits of microbial metabolisms.

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