BIOMASS PRODUCTION OF AZOTOBACTER

BY

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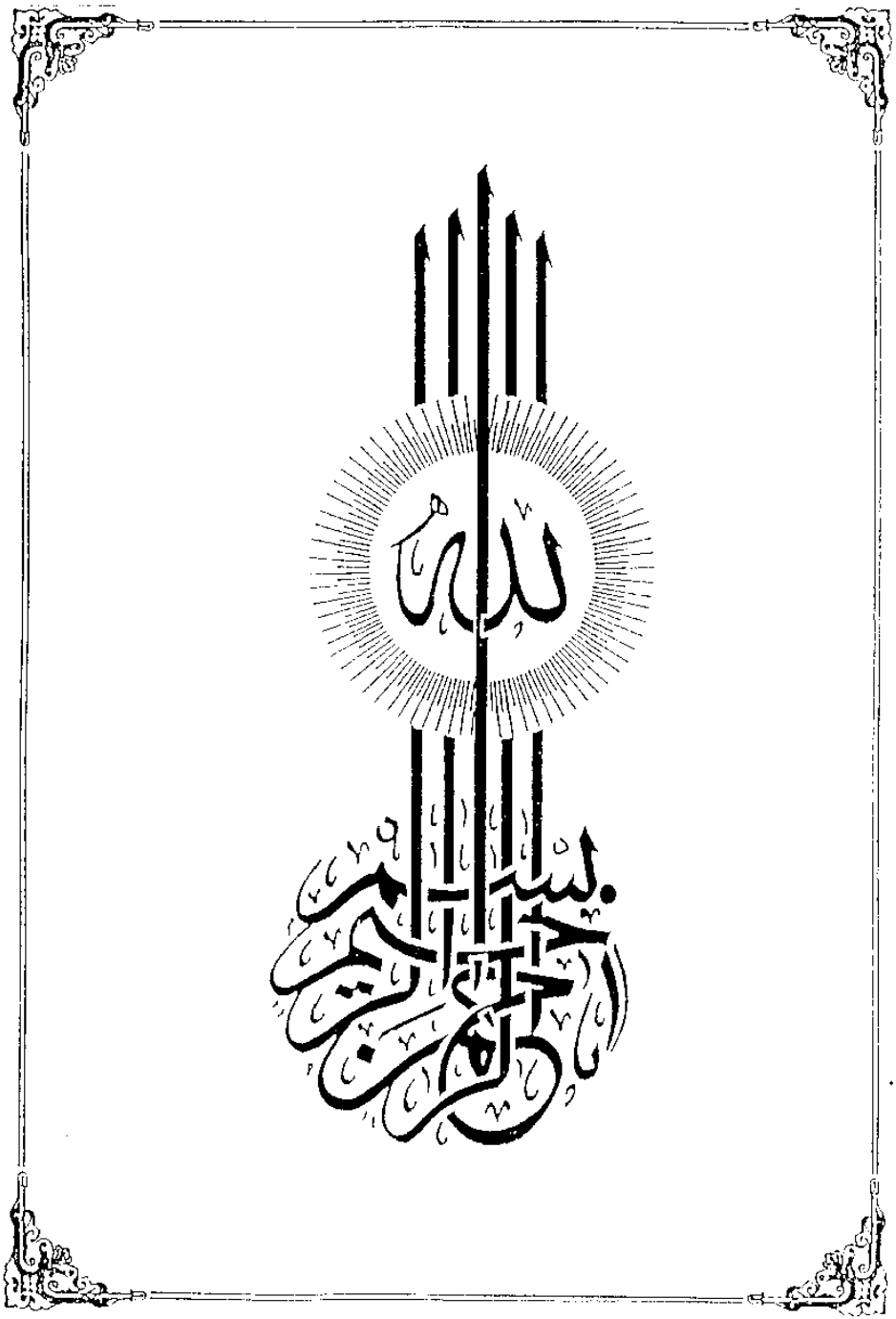

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INTRODUCTION

Nowadays, there is an increasing demand for the use of inorganic fertilizers in general and N-fertilizers in particular in developing countries to cover the agricultural requirements. The high cost of N-fertilizers especially when they are imported from abroad increases the cost of crop production. Moreover, the surplus use of N-fertilizers in agriculture are known to develop many environmental pollution problems. It is, therefore, important to explore alternative sources of nitrogen to partly meet the nitrogen requirement, and hence the use of biofertilizers was thoughy. These biofertilizers which can be more appropriately called microbial incculants in abroad since-may include inputs in agriculture which have manurial value and which are aided by microbial processes.

The discovery of legume fixation of atmospheric nitrogen in root nodules mediated by nodule bacteria was perhaps the first realization that microorganisms play a part in plant nutrition. With the exception of this example, there is an increasing tendency to use diazotrophs, i.e., Azotobacter and Azospirillum strains as biofertilizers fixing atmospheric nitrogen. Most of Azotobacter and/or Azospirillum inoculants -as far as we know-are applied in liquid culture which limits their application on large scale and reduce their viability by storage.

In Egypt, so many reports emphasize the presence of Azotobacter in soil and its efficiency in N_2-fixation. In view of these facts and the increasing demand for the use of N-fertilizers in agriculture, the present study was conducted as a trial for the biomass production of Azotobacter to be used as microbial inoculant fixing atmospheric nitrogen.

The study started with the evaluation of several Azotobacter strains to select the most efficient strains on the basis of N-fixation efficiency and growth parameters in a shake flask experiment. The biomass production of the most efficient strains as a mixed culture for the preparation of inoculants was carried out in fermentor as a batch culture. In this part of study, the effect of sugar concentration, pH, aeration and agitation on different growth parameters was studied in order to obtain optimal growth on large-scale cultivation. This was followed by the preparation of Azotobacter inoculants on different solid carrier materials. The survival rate of these inoculants at different storage temperatures and periods was investigated. Finally, the response of wheat plant to seed bacterization with the most efficient Az. inoculant with or without N-fertilization was evaluated in a pot experiment. If any positive response could be obtained by such practice, saving of a part of N-fertilizers could be gained.

REVIEW OF LITERATURE

The Azotobacteraceae is a coherent group of bacteria whose main characteristic is the ability to fix atmospheric nitrogen in a nitrogen-free or nitrogen-poor medium with organic compounds as an energy source (Rabotnova & Rodionova, 1953 ; Iswaran & Sen, 1960; Dalton & Postgate, 1967, 1969; Stewart, 1969; Gupta, 1972; Abd-El-Malek et al ,1975; Shawky, 1976 and Becking, 1981).

Members of this family are polymorphic showing seven different cell types. The cells characterizing these seven types are the following : a) oval cells in pairs, b) round cells in pairs, c) elongated cells in pairs, d) yeast like cells, e) small spherical cells in pairs, f) giant spherical cells in pairs and g) resting cells as cysts (Shawky, 1976).

Azotobacter species are also characterized by being Gram negative (although some may be Gram variable), motile, endospores are not formed, but some species form cysts (Ramanow, 1965; Abd-El-Malek and Ishac, 1966 and Becking, 1981).

Becking (1974) recognized four genera in the family Azotobacteraceae : Azotobacter, Azomons, Beijerinckia and Derxia. Tarrand (1978) added the genus Azospirillum to this family.

Becking (1974) and (1981) classified the Azotobacter into four species on the basis of the production of water-soluble flourescence, the ability to utilize

starch, mannitol and rhamnose as a sole source of carbon in nitrogen free medium, the motility and the type of flagella. These four species are : Az. chroococcum, Az. vinelandii, Az. beijerinckii and Az. paspali. The taxonomic key characters are cell form, motility, cyst formation, the G + C content (moles %) of the DNA. The formation of characteristic lipoid bodies (usually poly β-hydroxy butyrate) and catalase reaction.

Occurrence and role of Azotobecter in soil

It is well established that Azotobacter is universally distributed all over the world, in soils of all geographical regions. Azotobacter was isolated from the soil of Transval, East Africa and Egypt (Ashby, 1907), India (Hutchinson, 1912), Gava (Groenewege, 1913), Denmark (Weis and Barnebusch, 1914), Egypt, India, Japan, China, Syria, Hawaiian island, Gwatemala, Costa Rica, Spain, Italy, Russia, Mexico, Canada, Australia, Tahiti, Belgium, Queensland (Lipman and Burgess, 1915), USSR (Fedorov, 1957: Nepomiluev & Shishov, 1962), Southern France (Rouqueral, 1962), New Zealand (Di-Menna, 1966) and Iraq (Ishac et al, 1970).

In Egypt, many investigators revealed that Azotobacter is widely distributed in all Egyptian soils especially in the Nile valley (Ishac, 1958; Moubarek, 1960; Abd-El-Malek & Ishac, 1962; Taha et al , 1965; Vancura et al., 1965; Elwan & El-Sayed, 1967; Hegazi, 1969; Shady, 1970; Ibrahim, 1972; El-Safty, 1974; Omer, 1980; Girgis, 1985 and Sharaf, 1985).

The favourable effect of Azotobacter on yield of an agricultural crop is at present attributed to multiple action. Azotobacter can affect plant growth not only by fixing nitrogen but also by altering microbial balance, supressing pathogenic microorganisms, mobilizing soil phosphate and by providing metabolites that stimulate plant development as indole acetic acid and gibberillins (Ishac, 1958; Cooper, 1959; Moubarek, 1960; Abd-El-Malek & Ishac, 1962; Mishustin & Naumova, 1962; Taha et al, 1965; Vancura et al, 1965; Elwan & El-Sayed, 1967; Shady, 1970; Salahuddin & Aslam, 1971; Brown, 1974; El-Safty, 1974; Shende et al, 1975; Eweda, 1983 and Othman, 1986).

The production of heteroauxins (beta-indole acetic acid, gibberillins) and other related compounds by Azotobacter were reported by many investigators as Vancura et al, (1960) Jackson et al, (1964) and Elwan & El-Nagar (1972).

Barea and Brown (1974) stated that supernatant fluids of Az. paspali cultures contained indole-3-acetic acid, at least 3 gibberellines and 2 cytokinins.

Fouad (1981) reported that Azotobacter and Azospirillum produced considerable quantities of indole acetic acid (IAA) which stimulate the expousion.

Eweda (1983) reported that Az. paspali was active in IAA and gibberelline (GA_3) production. It producted 1.4 mg and 0.45 mg ml^{-1} medium respectively.

Factors affecting growth and N_2-fixation of Azotobacter

So many factors were reported to affect the growth and N_2-fixing ability of Azotobacter (Becking, 1962; Dalton and Postgate, 1969 and Alexander, 1977). The nitrogen fixing capacity of Azotobacter may vary considerably depending on the conditions of cultivation of the strains, i.e., the composition of the nutrient medium, its acidity Torosvik, 1973), temperature (Iswaran & Sen, 1960), aeration (Dalton and Postgate, 1969 , carbon source (Bahadur and Lripathi,, 1976), trace elements (Krylova, 1963) and moisture (Alexander, 1977).

a. pH

Yamagata and Itan (1923), Stapp and Ruschmann (1924) and Jensen (1954) noted that the optimum pH for Az. chroococcum and Az. vinelandii was near pH 7.5 and the limit for visible growth in liquid or solid media was near or slightly below 6.0.

Yamagata and Wilson (1923) indicated that the reaction of the soil is important in determining what type of Azotobacter might be present. The pH of the medium producing the greatest increase in cells was 6.6 for the beijerinckii type 6.8 for the chroococcum type and 7.0 for the vinelandii type

Peterson (1925) observed no macroscopic growth of Azotobacter chroococcum in nitrate medium below pH 6.0.

Burk et al (1934) and Csaky (1949) found in manometric experiments that growth of Az. chroococcum and Az. vinelandii ceased abrupty at pH 6.0 with free nitrogen. While with nitrate, ammonia and urea it continued at a reduced rate and did not appear to cease until pH 4.0-4.5, but this observation had not been generally confirmed.

The endogenous respiration of Az. chroococcum according to Harris and Gainy (1944) had an optimum at pH 7.0 and continued in a slight growth at pH 5.5-5.8, where it was somewhat stimulated by calcium ions.

Blinkov (1951) noted that the optimum pH for Azotobacter lied within the pH range from 7.2 to 8.2.

Koleshko (1961) showed that acidification of the medium to pH 5.3 inhibited propagation of Azotobacter much more strongly than alkalification to pH 9.0.

Paul and Newton (1961) claimed that Azotobacter chroococcum was capable of initiating growth between pH values of 6.2 and 7.9 with an optimum growth rate at pH 7.0. Azotobacter did not develop below a pH of 6.2.

El-Safty (1974) found that the pH range for growth of Azotobacter strains generally lied between 6.9-9.5. He also added that nitrogen fixation was nil below pH 6.5 and at pH 9.5.

Shawky (1976) noted that the sensitivity of Azotobacter to acidity was observed when the organism was grown on agar medium of different pH values. Optimum range for growth lied between pH 7.0 and 8.0 although the organism could withstand pH levels between 6.5 and 9.5.

Considering the effect of soil pH on the growth of Azotobacter spp, Gainey (1923) reported that the absence of Azotobacter in some soils was related to acidity exceeding pH 6.0.

Burk et al (1934) noted that the pH level 6.0 inhibited population and N_2-fixation of Azotobacter. He also found that the growth of Azotobacter was vigorous in alkaline soils. The limiting alkaline reaction was reported to be below pH 9 or 10.

Jensen (1965) reported that the Azotobacter sp grew efficiently and fixed nitrogen in soil at pH 8.7-9.

Hegazi (1969) observed that the high Azotobacter densities were most frequently found in soils with pH 7.8.

Torosvik (1973) studying the presence of Azotobacter in Norwagian soils, found that only six samples contained Azotobacter from 28 samples having pH 4.1-7.7, and predominant species were Az. chroococcum and Az. beijerinckii.

b. Temperature

Abd-El-Malek (1971) reported that optimal activity of Azotobacter as well as clostridia was found to be around 30°C. Higher temperatures favoured clostridia while lower ones favoured Azotobacter.

Larson and Neal (1978) mentioned that nitrogen dependent anaerobic growth occurred over a temperature range of 10-35°C. Optimum rate of growth was 30 °C, while the greatest production of cells occurred at 20 °C.

Skujins and Klubek (1978) showed that in soils of semi - arid regions the highest rate of fixation was in mid-morning, when temperature was relatively cool and the soil surface was moist from dew. As the temperature increased the N_2-fixation increased until it reached a certain maximum, thereafter decreased. Optimum N_2-fixation (C_2H_2 reduction) occurred between 19 °C and 23 °C.

c. Aeration

Meyerhof and Burk (1928) reported that high oxygen tension inhibited the growth of Azotobacter. The respiration reached its maximal at Po_2 = 0.15 - 0.17 atm and intensity of respiration decreased with the increase or decrease of Po_2. They found a negative influence that an increase in the partial tension of oxygen had on the nitrogen fixation by Azotobacter. Complete inhibition of N_2-fixation occurred at