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PROGRAMME MIRCEN

NITROGEN FIXATION IN LEGUMES

Part 1. RHIZOBIUM INOCULANTS

Part 2. ROLE Of MIRCEN

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Part 1. RHIZOBIUM INOCULANTS

INTRODUCTION

1 - Nitrogen cycle (Fig. 1)

Leguminous crops play an important role as a supplier of protein for third world people. In many cases, soils lack available nitrogen which is a major limiting factor for plant growth and yield.

However, plants can not Fix the free air nitrogen without the help of effective Rhizobia in the root systems nodules (1). Nitrogen issued from Plant residues or animal wastes (2) is mineralized (3) before its assimilation by plants under NH_4^+ or NO_3^- form (4). In natural conditions, plant growth depens on microbial activities in the soil. Even through there is a heavy dressing of fertilizer from industriel fixation (5), soil microorganisms contribute ta increase amount of nitrogen assimilated by plants.

Soil NH_4^+ and NO_3^- content intervene in four process :

i) Ammonia and nitrate production : ammonia production (3) is a stage of ths mineralization. Different hetcrotrophic microorqanisms contribute at its production. However, specific autotrophic bactoria produce the nitrate (6).

$NH_4^+ + 3/2\ O2 \longrightarrow NO_2^- + 2H^+ + H_2O$ (Nitrosomonas)

$NO_2^- + 1/2\ O2 \longrightarrow NO_3^-$ (Nitrobacter)

ii) The reorganization (7) : Use of the mineral nitrogen NH_4^+ by heterotrophic bacteria for their proteinic synthesis.

iii) The colloidal solution of the clay-humus complex play an important rol in tha ammonia absorption (8) and the chomical fixation (9).

iv) The nitrogen wastes by volatilization (10), lixiviation in soil (11) and chemical or biological denitrification (12).

2 - Biological nitrogen fixation

plants of leguminous and actinorhizal crops fix the free air nitrogen modiated by Rhizobia. This process is callod Biological Nitroqon Fixation (BNF) : housed in plant nodules, Rhizobia obtain food and energy from their host ; Rhizobia in turn, fix atmosphsric nitrogon which is thon metabolized by tho plant host.

It is well astablished that the nodule is a structure iesued from the proliferation of cortical root cells infected by Rhizobia. Insids the nodule, the bacteroids are enclosed within membrane envelopes and are tho site of nitrogon fixation.

Nitrogen fixation is an anaerobic process and tho leghaemoglobin regulates the oxygen. According to following diagram (Fig. 2), nitrogenaso is the enzyme which mediates the reduction of N_2 to NH_3. This enzyme is made up of two components : one with both iron (Fe) and molybdenum (Mo) and the second with iron (Fe) only. The first stable product in BNF is ammonia. The ammonium ion NH_4^+ procod is assimilated through GS-GOGAT (Glutamine Synthatase-Glutamate synthetase) pathway for aminoacids and proteins synthesis.

Therefore, BNF permits plant growth without having recourse to expensive chemical fertilizers. It is a true nitrogen economy. This explains why many countries, especially the developing countries, make an effort for promoting and improving BNF by culturing *Rhizobia* in laboratories and producing *Rhizobium* inoculants.

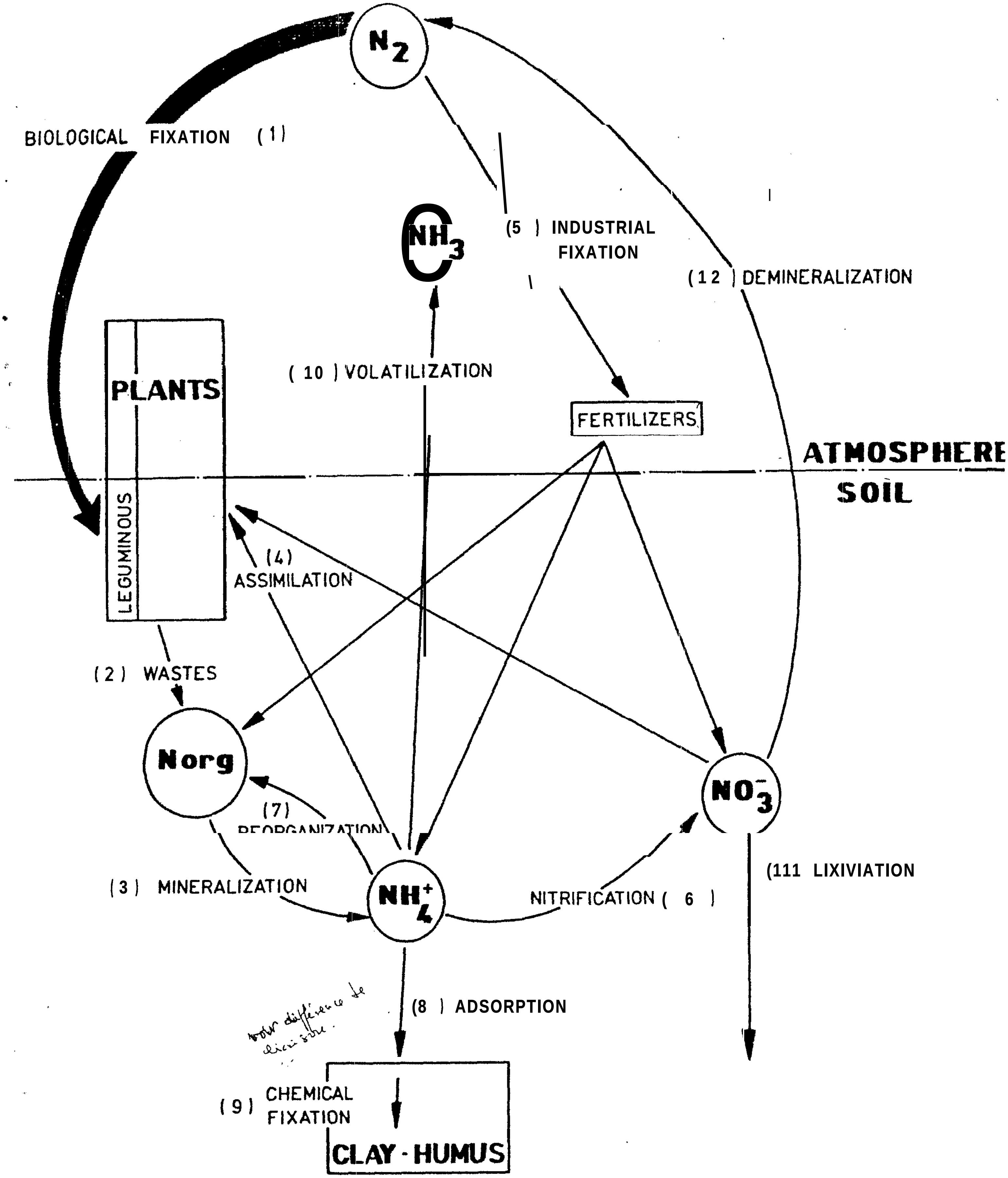

FIG. 1

NITROGEN CYCLE

CURVED ARROWS ( ) BIOLOGICAL PROCESS

STRAIGHT ARROWS ( ) CHEMICAL PROCESS

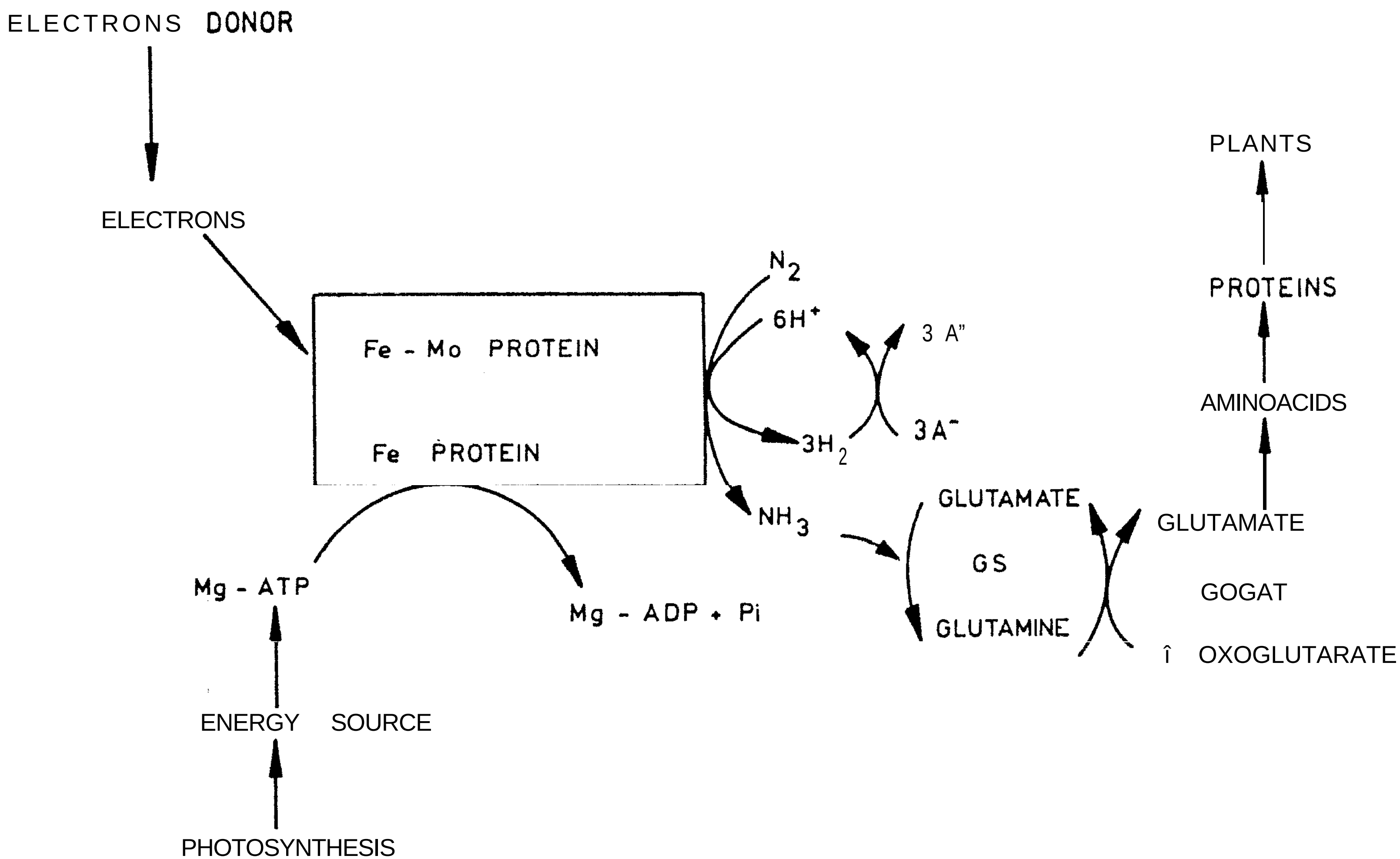

FIG. 2

SCHEME FOR BNF IN A ROOT NODULE

A = ELECTRONS ACCEPTOR

B.E/M

1 - Rhizobium strain selection

The first step in legume inoculant manufacture is to obtain effective nitrogen fixing strains of Rhizobia for the legumes to be inoculated. The attributes needed in an inoculant strain are :

(i) the ability to form nitrogen fixing nodules on the host for which it is recommended,

(ii) the high competitiveness in nodules formation and

(iii) the survival and the multiplication of Rhizobia in soil (DATE, 1976).

1.1 - Nitrogen fixing nodules formation

The effectiweness of a Rhizobium strain is its ability to fix nitrogen within the nodules. The effectiveness is influenced by intrinsic factors (variations within a cross inoculation group) and extrinsic factors (environmental conditions : pH, varying soil types, soil nitrogen level, interactions with indigenous soil microorganisms).

1.2 - Competitiveness of Rhizobia in nodule formation

An important characteristic in strain Rhizobium selection is the ability of a strain to successfully compete for nodule formation with the native rhizobial population which is generally less effective.

1.3 - Survival of rhizobium

The most effective and most competitive Rhizobium must multiply and survive within soil.

2 - Cultivation and massive production of Rhizobia

2.1 - Cultivation of Rhizobia

Rhizobia grows in a routine medium whose composition is given in the appendix. Rhizobia can be cultured in shake culture or in glass bottles, or in any of a wide variety of fermentes. Monosaccharides and disaccharides are the most common source of carbon for Rhizobium isolates although some slow growing strains prefer pentosee (arabinose) instead of mannitol or sucrose (DATE, 1974).

2.2 - Massive production of rhizobium

To obtain a Rhizobium culture of a high concentration, the fermentor is used. Prior to the cultivation of Rhizobium in a fermentor, it is usually recommended to start, first, a preliminary culture in a small flask. Later, the starter culture is then transferred aseptically to the production fermenter at the rata of 1 % by volume. Culture is aerated by sterile air. An oxygen partial pressure of 0.15 atmosphere is optimum and Rhizobia grow best in the range of 25 to 32°C.

There are different types of fermenter : from large fermentors with complicated devises (BURTON, 1967) to simple fermentors used in Australian (DATE, 1974), (Fig. 3). According to australian experience, autoclavable fermentation

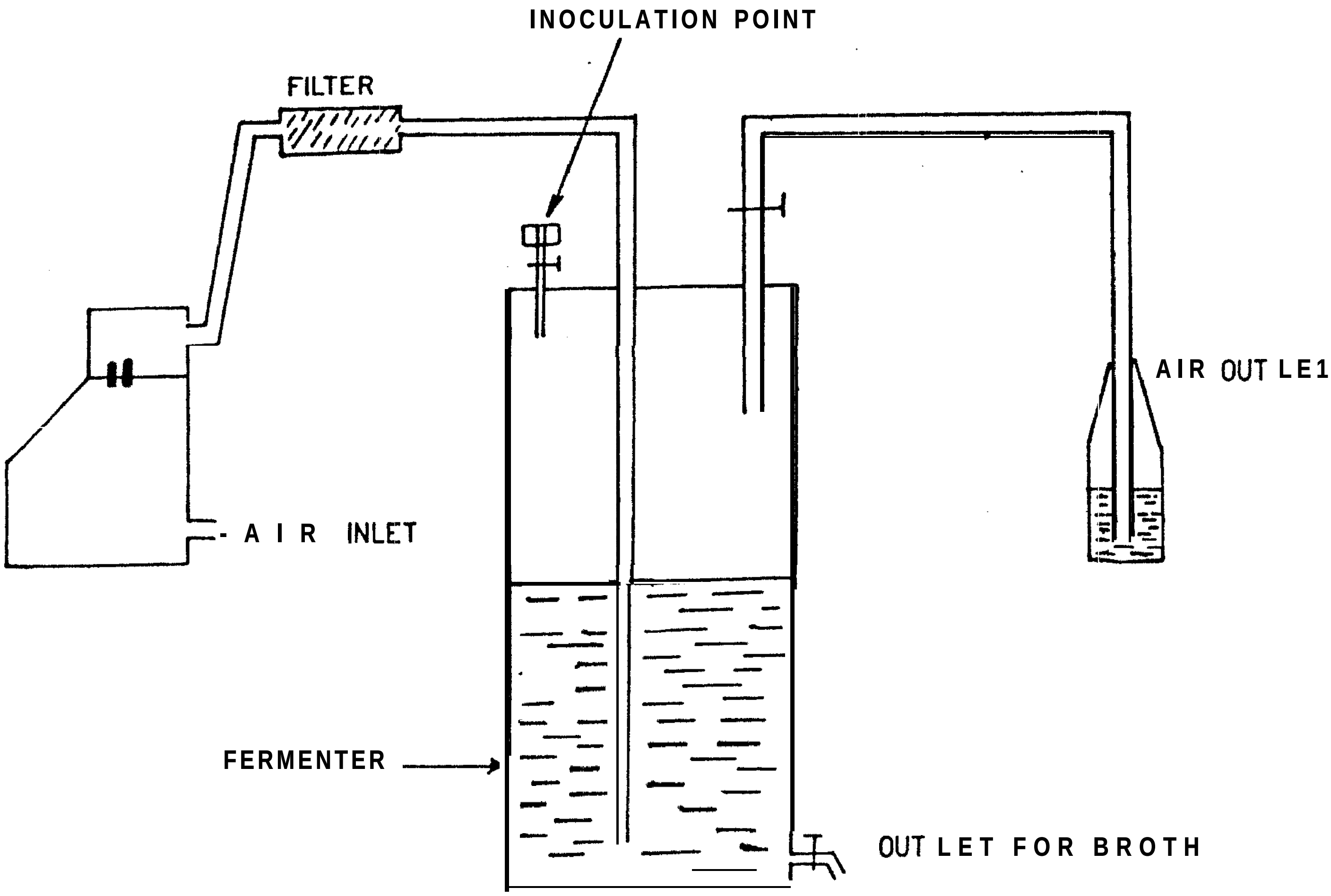

Fig. 3

DIAGRAMMATIC REPRESENTATION OF THE SIMPLE AUSTRALIAN FERMENTER FOR RHIZOBIUM, (DATE 1974)

unit is better tharr self operating complicated fermenters requirincj steam sterilization.

3 - 1 noculant carriers

The qualities of a good carrier inoculant material are : (1) highly absorptive and easy to process ; (2) Non-toxic to Rhizobia ; (3) Easy to sterilize ; (4) Available in adequate amounts ; (5) Inexpensive and (6) good adhesion to seeds (BURTON, 1981).

Many materials can be used as carriers : peat, charcoal, clay, lignte, compost, straw, pulverized minerals such as vermiculite, and polyacrylamido (DOMMERGUES et al., 1973).

Peat has boen the most commonly used base for commercial inoculants. It is agreed that as an inoculant base, sterile peat is superior tc non sterile peat and that heat treated or autoclaved peat is better than air dried peat.

4 - Quality control

Thc aim of Rhizobium inoculant quality control is to avoid problems during production and storage and also to ensure the high quelity cultures. Howvever, it is necessary to standardize the inoculant. For a particular local conditions, th, number of viable cells in Rhizobium inoculant standard must be superior to just adequate number of Rhizobium for nodulating thc legume in sterile conditions. Within the limits of inoculum technology (i.e. tho many factors which affect inoculant's quality), crop agronomic caractaristies determine inoculant standard. Many researchers recommended an inoculum which would providc approximatively 10,000 to 100,000 viable Rhizobia per seed for a good inoculation (table 1).

'Table 1 : Number of viable Ahizobium cells required per seed.
(BORDELEAU, L. and PREVOST, D. 1981).

Crop	Seed size (Number seeds/kg)	Number of viable cells per seed
Alfalfa Clover Bird's-foot trefoil	Small >200,000	10^3
Sainfoin	Tntermediate 50,000 - 200,000	10^4
Beans Peas Soybeans	Large <30,000	10^5

5 - Agronomic importance

Inoculation successes of failures in tho field have been shown (HAM et al., 1976 ; BALASUNDARAM and SUBBA RAO, 1977; SUBBA RAO, 1976).

Failures to obtain the desirod response may be due to (i) the presence than introduced effective Rhizobia strains, (ii) the presence of antagonistç of Rhizobia which minimizc the number of Rhizobia in the rhizophere and (iii) soil conditions which limit symbiosis : acidity, alkalinity, high nitrogen leval in soil or othar factors.

The use of Rhizobial cultures in legume establishment has been widely rwcognized, especially in areas where indiganous Rhizobia have beon found to be inadequate.

CONCLUSION

1 n many countries, especially in the developing countries, grain legume is produced mainly by small farmers who use multiple cropping systems and minimal technical imputs. The essential role of thc Microbiolugical Resources Centers (MIRCEN) is to promote the uso of inoculant technology in these developing countries (see part 2).

PART 2 : ROLE Of MIRCEN

ROLE OF MIRCEN IN DEVELOPING COUNTRIES

Developing countries are today facing with many difficulties as insufficiency of food, environmental pollution, energy crisis. In some of these countries, hunger abats social and political unrest.

In the present fact, science of microbiology offers a wide field of applications of microbiological technologies in the developing countries because it takes actions in agricultural productivity, food and energy production, health, etc...

In food production, single cell protein (SCP) has attrncted considorable interest as a group of fuods rich in protein, producsd by non-agricultural means and hopefully less oxpensivo than convantional protein-rich materials such as soybean meal. Use of SCP is yet limited. It is uscd for animale nutrition. Tho micrcarganisms ussd for SCP production include bactaria, yeast and algae.

Wood is the more important combustible usod in developing countries. <u>Frankia</u> is an actinomycot which can nodulate <u>Casuarina equisetifolia</u>, a nitrogen fixing tree.

In the other hand, one of the important biological research is the production, of cooking gas from agricultural wastes.

Soil and mater pollution require microbiological technologies : effluent water coming from factories must degreted through microbial actions to avcid water pollution.

Through microbial activities, we can obtain antibodies, Indispensable enzymes like protease, amylase, cellulase and vitarnins which are all important to our daily living.

Metabolits produced by soil microorganisms improve plant groth. Crctp yield can be improved by domesticating biological nitrogen fixation in order to reduce the use of expcnsiva nitrogon fertilizers.

Science of microbiology is also applicd for protecting crops against dieeases mediated by <u>Baccillus thuringiansis</u>, <u>Pseudomonas septica</u>, <u>Clostridium perfringens</u> and other entomopathogen microorganisms.

These examples illustrate the role of microorganisms in thu development of third world countrias and tho importance of culture collection of micruorganisms. This is why UNEP/UNESCO/ICRO panel on microbiology has launche the Microbiolugical Resources Center (MIRCEN) programs which are designed to :

a) - Provide the infrastructure for a world network which would incorporate rogional and interregional co-operating laboratories geared to the management, distribution and utilization of thc microbial gene pool ;

b) - Reinforce efforts relating to the conservation of microorganisms, with emphasis on _Rhizobium_ gene pools, in developing countries, with an agrarian base ;

c) - Foster thc development of now inoxpensive technologies rative to the region ;

d) - Promote the applications of microbiology in ordor to strengthen rural economies ;

e) - Serve as focal centers for the training of manpower and the diffusion of microbiological knowledge.

In Senegal, the ISRA (1) agriculture1 ond forestry resoarch centers at Bambey and Dakar-Hann, respectively, have been dosignated as the West Africa MIRCEN. That MIRCEN will focus on establishing a regional microbial culture collection to serve the agricultural production.

Important species of microorganisms of economic significsncs, which are usod in ths preparntion of biofertilizer inoculum production (for example, Rhizobium, Frankia, Myc orrhiza), and bioonergy goneration (for example, biogas) will be centralized for prossrvation and use primarily in Senegal. At a later stage, co-opnrating laboraties in neighbouring countries of Mali, Upper Volta, Gambia, Guinea, Niger, Chad and Togo will benefit from the culture collection at the MIRCEN.

Tha MIRCEN will also undertake collaborative research projects, recommend regional fellowships, organize short- term training and eventually attract international contracts for research work in the region.

(1) ISRA : Institut Sénégalais de Recherches Agronomiques.
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Appendix : Routine medium for growth of <u>Rhizobium</u>

Yeast-Extract Mannitol broth.

K_2HPO_4	0.5	g
$MgSO_4, 7H_2O$	0.2	g
NaCl	0.1	g
Mannitol	10	g
Yeast-extract	1	g
Distilled water	1000	ml
Agar	15	g

pH = 6.8