भारतीय मानक

मृदा में जैव-उर्वरक के रूप में नीली-हरित शैवाल के अनुप्रयोग की रीति संहिता

Indian Standard

CODE OF PRACTICE FOR PREPARATION AND APPLICATION OF BLUE-GREEN ALGAE AS BIOFERTILIZER IN SOILS

ICS 65.080

BUREAU OF INDIAN STANDARDS
MANAK BHAVAN, 9 BAHADUR SHAH ZAFAR MARG
NEW DELHI 110002

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FOREWORD

This Indian Standard was adopted by the Bureau of Indian Standards, after the draft finalized by the Soil Quality and Improvement Sectional Committee had been approved by the Food and Agriculture Division Council.

The microbial denizens of soil play an important role in nutrient mobilization. The nitrogen fixing *blue-green algae* (BGA) which commonly occur in moist waterlogged fields form an important component of the soil micro-organisms and have been held responsible for the spontaneous fertility of rice soils. Waterlogged conditions, high humidity and temperature and diffused light under the crop canopy in paddy fields favour their proliferation. However, algalization effect may vary depending on the region, rate of growth, stress compatibility and sporulating capacity.

BGA grow well in neutral to alkaline soils having *p*H range 6.5 to 8.5. They may also be grown in acidic soils after proper liming. The BGA inoculation increases the availability of nitrogen in the soil. They add organic matter through the oxygen, liberating process of photosynthesis and their polysaccharidic sheath binds the soil particles. These activities improve the physical and chemical properties of the soil which is reflected in the form of reduced compaction and oxidizable matter content. The hormone like substances excreted by the algae, enable the crop plants to utilize more of the applied nutrients. They show pronounced supplementation effect at lower levels of fertilizer nitrogen. Use of BGA may add 15 to 25 kg nitrogen/hectare/season.

The strains of BGA have to be selected on the basis of their stress compatibility, growth and nitrogen fixing capacity response to temperature. Strains suitable for defined habitats and requirements can be developed through screening of the natural populations. Studies conducted under different agroclimatic conditions have shown, That forms like *Aulosira, Calothrix, Scytonema* and *Tolypothrix* are better suited for upland and rainfed paddies. Perpetually waterlogged rice crop responds better to inoculation by *Anabaena, Nostoc, Cylindrospermum* and *Hapalosiphon.*

A need was, therefore, felt to formulate Indian Standard on the subject stipulating code of practice for preparation and application of blue-green algae as biofertilizer in soils for the benefit of processers and the users of the product. In preparation of this standard considerable assistance has been derived from the National Facility for Blue-green Algal Collections, Indian Agricultural Research Institute, New Delhi.

For the purpose of deciding whether a particular requirement of this standard is complied with, the final value, observed or calculated, expressing the result of a test or analysis, shall be rounded off in accordance with IS 2 : 1960 'Rules for rounding off numerical values (*revised*)'. The number of significant places retained in the rounded off value should be the same as that of the specified value in this standard.

Indian Standard

CODE OF PRACTICE FOR PREPARATION AND APPLICATION OF BLUE-GREEN ALGAE AS BIOFERTILIZER IN SOILS

1 SCOPE

This standard prescribes the code of practice for preparation and application of blue-green algae as biofertilizer in the soils.

2 INOCULUM PRODUCTION

2.1 Strain Selection

This will be the main activity of the Research and Development wing of the Algal biofertilizer unit involving regular screening of the naturally occurring algae for selecting the desired organisms. The criteria for screening should be:

i) Growth rate,
ii) Nitrogen fixation,
iii) *p*H and salinity tolerance, and
iv) Response to agrochemicals like fertilizers and pesticides.

Mucilage formation and sporulation will be the additional attributes.

2.2 Culture Maintenance

The selected algal strains will be maintaind on agar slants containing appropriate media in unialgal state. The often used strains are maintained in liquid media also, for immediate use in the process of inoculum scale up.

Cool daylight, 40 watts fluorescent tube lamps are used to provide a light intensity of about 2 500 lux and the temperature is maintained at about 30°C. Cultures on agar slants are more conveniently maintained in 15 ml capacity, backelite screw cap with rubber liner, culture tubes. Mother cultures in these culture tubes are maintained in the stock culture room at about 500 lux light intensity and 20°C temperature.

2.3 Culture Medium

Since the nitrogen fixing blue-green algae are photolithotrophs and use the nitrogen from the air, simple inorganic medium without any nitrogen source is employed for maintaining and growing their cultures. While the laboratories may develop different suitable media, generally the following medium is used for purpose.

2.3.1 *Medium Composition*

2.3.1.1 *Constituents*

	ml/g/l
Di-Potassium hydrogen phosphate	0.2
Magnesium sulphate	0.2
Calcium chloride	0.1
A-5 Micronutrient solution	1.0
Fe-EDTA Stock solution	1.0
*p*H	7.0

2.3.1.2 *A-5 Micronutrient*

Boric acid	2.86
Manganese chloride	1.81
Zinc sulphate	0.222
Sodium molybdate	0.017 7
Copper sulphate	0.079
Fe-EDTA Stock solution	

2.3.1.3 *Fe-EDTA Stock Solution*

Dissolve 26.1 g of ethylene diamine tetra acetic acid (disodium salt) in 268 ml of 1N potassium hydroxide (56 g/l w/v) solution. Make up the volume to 1 litre. Aerate the solution overnight to produce stable complex marked by the development of dark brown colour. Make up the volume again to one litre. One ml of this stock solution in 11 gives 5 ppm of iron.

2.4 Scaling Up of the Inoculum

Inoculation of the raceways is done by using the scaled up culture of the algae grown under growth room conditions. The ratio between the volume to be inoculated and the inoculum should be 10:1. The required amount of the inoculum is produced as per the following flow chart:

Slant culture

Conical flasks

Aspirator bottles

Natural, transparent glass carboys

The culture in the carboys is allowed to grow under controlled conditions mentioned earlier at **2.2** for about 20 days or till it attains the optical density (OD) of 0.6 at 550 nm. This culture is used to inoculate the raceways. Upto the aspirator bottle stage, the cultures

are grown in sterilized medium under aseptic conditions. At the carboy stage, boiled water is used for preparing the medium. All the cultures under laboratory conditions are maintained in unialgal stage.

3 PROCESS OPTIMIZATION

3.1 Raceways

Large scale multiplication of the selected algae is done in raceways lodged in a covered space. Each alga is grown separately. A raceway is a shallow pond of about 35 cm depth with round corners. The length can be varied depending upon the availability of land but the width should not be more than 3 m. The pond is divided by a central wall, leaving 1 m space at either ends. In one of the channels so formed is fitted a paddle wheel of appropriate size, energized by an electric motor. This is used to agitate the culture in the raceway so that it races through the channels. This ensures unifrom distribution of the nutrients and light through the culture and does not allow settling of the algal biomass.

Lodging of raceways in a covered space ensures round the year production and provides protection against contamination and attack by the parasites and predators. It also accelerates the algal growth and enables complete control on the growth conditions and harvesting of the culture at the desired growth stage.

3.2 Growing Algae in the Raceways

Normally, in the raceways, the nutrients are used at half the recommended strength. The culture is allowed to grow till it attains the optical density of 1.0 to 1.5 at 550 nm. After this, daily harvesting of the algal culture is done by filtering 1/10 of the total volume of the culture in the raceway. The culture is filtered through cheese cloth and the filtrate is drained back into the raceway. The growth rate of the alga is regularly monitored as it tends to slow down with the depletion of the nutrients. Whenever needed, appropriate quantities of the nutrients are added to the raceway.

3.3 Productivity of the Raceway

Under ideal conditions of 30 to 35°C temperature and 4 to 5 K lux light intensity, the productivity of the raceway comes to about 100 g fresh algal biomass/m^2/day.

3.4 Preparation of Dry Algal Inoculum

The harvested algal biomass is wet-mixed in equal quantity with a locally available clay known as '*Multani mitti*', Fuller's earth, which is mainly montmorillonite. It has a very high water holding capacity and practically does not have any microbial denizen. The paste is dried under hot air current at around 60°C or even at ambient temperature and the dried flakes are powdered to 200 mesh. This makes a very good quality inoculum and 0.5 to 1.0 kg of this is sufficient to inoculate one acre area.

The inoculum so prepared has the colony forming units cfu value of about 10 000/g of the inoculum and does not show any appreciable loss in the cfu even after storage for 2 years.

3.5 Quality of the Inoculum

The quality of the algal inoculum may be quantified in terms of colony forming units (cfu). An ideal algal inoculum should have a cfu value of at least 10 000. Shelf life of the inoculum shall also be declared by the manufacturers.

4 FIELD APPLICATION

4.1 The recommended method of application of the algal inoculum is broadcasting on standing water, about 3-4 days after transplantation. The following care shall be taken during application of BGA:

i) Since the size of the inoculum is too small to ensure uniform distribution over an area of one acre, the inoculum can be mixed with clean and sieved soil and then broadcasted.

ii) Chemical fertilizers and algal inoculum should not be mixed and applied separately.

iii) After the application of the algal inoculum, the field should be kept waterlogged for about.

iv) Heavily fertilized rice fields generally show profuse growth of green algae which act as weeds and also reduce tillering in the rice plants. These can be differentiated from the blue-green algae by their grass green colour and fibrous nature. The green algae turn dark violet when treated with iodine but the blue-green algae remain unaffected. The green algae can be removed manually and burried in a pit and if their growth is intense, copper sulphate @ 4.0 kg/ha may be used which will selectively kill the green algae.

4.2 Storage

4.2.1 The dried clay based algal inoculum packed in polythene bags can be stored at room temperature in a dry place.

4.2.2 The packets should be kept away from fertilizers and pesticides.

4.2.3 The packets may be stored for two years without any loss in the viability of the inoculum.

5 CONSTRAINTS

5.1 Heavily fertilized rice fields show initial dominance of green algae even after algalization. These are replaced by the inoculated blue-green algae as the fertilizer nitrogen is consumed.

5.2 The effect of BGA biofertilizer is not as instantaneous as in the case of inorganic fertilizers. It is slow but sustained and additive.

5.3 Establishent of the algal inoculum in the field may be seen in the form of floating algal biomass on the surface of the water or as numerous small, glistening air bubbles adhering to the soil surface. These indications are best seen in the afternoon.

5.4 After about 15-20 days of inoculation, the rice plants in the algalized plots appear greener than the non algalized plots.

5.5 Algalization induces early grain setting and maturity which is indicated by the early drooping down of the ear heads of the treated plants.

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