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OPTIMIZATION OF AN INDUSTRIAL PROCESS FOR OBTAINING AN ACTIVE
SOIL-IMPROVER BIOMASS (BIOLOGICAL FERTILIZER)

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OPTIMIZATION OF AN INDUSTRIAL PROCESS FOR THE OBTAINING OF AN ACTIVE
SOIL-IMPROVER BIOMASS (BIOLOGICAL FERTILIZER)

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ABSTRACT

A description is given of the different experimental phases developed for the refining of an industrial process which makes it possible to obtain a high percentage of active biomass, as cheaply as possible, with biological capacity for improving soil fertility.

The starting point for the development of this process was the observation of the enhancing effect on the growth of microbial populations of the incorporation of free amino acids in elemental culture media. The results are given as obtained in the culture of a species of Rhizobium, as example, at the laboratory level.

A description is given of the technology and electronic elements used in the industrial phase for controlling the parameters and physical, chemical and biochemical values of the fermentation.

Studies and experiments have been carried out with different organic substrats of plant origin, and whose cellulose, hemicellulose and lignin contents are given in Table III.

A list is given of some of the strains of microorganisms selected. The inclusion of a free amino acid complex in the culture media and the industrial process, forming part of specific biocatalysts, makes it possible to obtain a commercial product which, based on cheap organic substrats, maintains very high micro-organism populations, with positive effects on the soil micro-flora, in the order of 10^{15} viable microorg./g.

On the basis of this configuration, an industrial plant and anexés was designed with capacity for the treatment of 20,000 metric tons of organic substrats peats or agricultural residues) and to obtain an output of active biomass/biological fertilizer of 12,000 metric tons/annum.

Finally, the influence is shown of these products on soils, and their repercussion in agricultural production.

This work have the purpose to justify a find of great importance for the microorganisms of the soil. That is, the incorporation of free aminoacids to cheap culture media, useful for the microbial biomass production; which, by means of classic biotechnological methods, is submitted to an enrichment, reinforced, treated and kept in polyethylene bags of 50 kg each; so than it can effect in the soil a favourable action on the structure and the agricultural yields. The industrial production of the "active biomasses" or "biological fertilizers" haven't appearance as a novelty; but the importance of the specific results of the product which is described here and the points about the technology of the processes, justify the existence of this paper, supported by a commercial activities of several years in four countries.

For growth, micro-organisms required assimilable carbon and nitrogen, mineral salts and a series of minor elements called growth factors. In the right conditions of aeration, temperature, pH, osmotic pressure and absence of inhibitors, a microbial population can develop following a type of characteristic conduct which is perfectly quantifiable and can be adjusted to theoretical models.

Keeping this in mind, we try to rise the process from laboratory level to pilot plant and industrial production. The optimization of the yields set up a variety of problems with unavoidable and necessary resolution.

- 1) The search, preparation and use of proper substrats.
- 2) The maintenance preparation and swing of the starters on the substrats, maintaining the optimal conditions of viability for growth.
- 3) The addition of biocatalysts containing the amino acid complex and other complementary nutritive substances.
- 4) The inherent difficulties of a batch-type fermentation.
- 5) The optimum volume of degradable substrat in each cell in order to sustain the guaranteed level of active microbial population in the final product and until the limit of the period of activity which is indica-

ted on the commercial packing.

6) The re-design and improvement of the original fermenters, as soon as the services, the accessories, instrumentation and control

7) The quality control of products, at the laboratory and the field tests. Control is required also for the old test, defective products and inadequate use. It's very interesting to pick up the reports of experimented users.

USE OF FREE AMINOACIDS: GROWTH OF RHIZOBIUM LEGUMINOSARUM biovar TRIFOLII

This is a very simple experiment, without adjustments to mathematical models, where the aim is to observe the effect of free amino acids incorporated in to a standard culture medium for Rhizobium.

Strain was used from the laboratory collection, extracted from clover nodules and incubated in a standard liquid medium which is described below (Table I) (M-1). As is the usual practice, this medium contains mannitol to difficult contamination, and tryptophan as growth factor. The yeast extract is a source of vitamins.

Incubated with orbital agitation, at 30° and 200 r/min, very favorable values were obtained, of 1.2×10^9 U.F.C/ml, at 120 hours (see fig. 1.)

The biomass estimate was carried out using a variety of methods, simultaneously; they revealed no parallels, and the maximum values found were the ones taken. a) Germ count using the MPN method. b) Plate coun. c) Microscopic Direct count. d) Optical density.

The first two were done on the same culture medium, liquid and solidified with agar at 1.75%.

In the new conditions the M-1 culture medium was replaced by M-2, eliminating the yeast extract and incorporating the free amino acid complex. One of the flasks containing the microbial population 1.2×10^4 UFC/ml was used to inoculate a volume 12 times greater, from a fermenter where a stationary culture (batch) experiment had been carried out. Agitation, pH and temperature were maintained, with a aeration at 1.5 l/min. Samples were taken every 5 hours and processed in the same way. Direct count

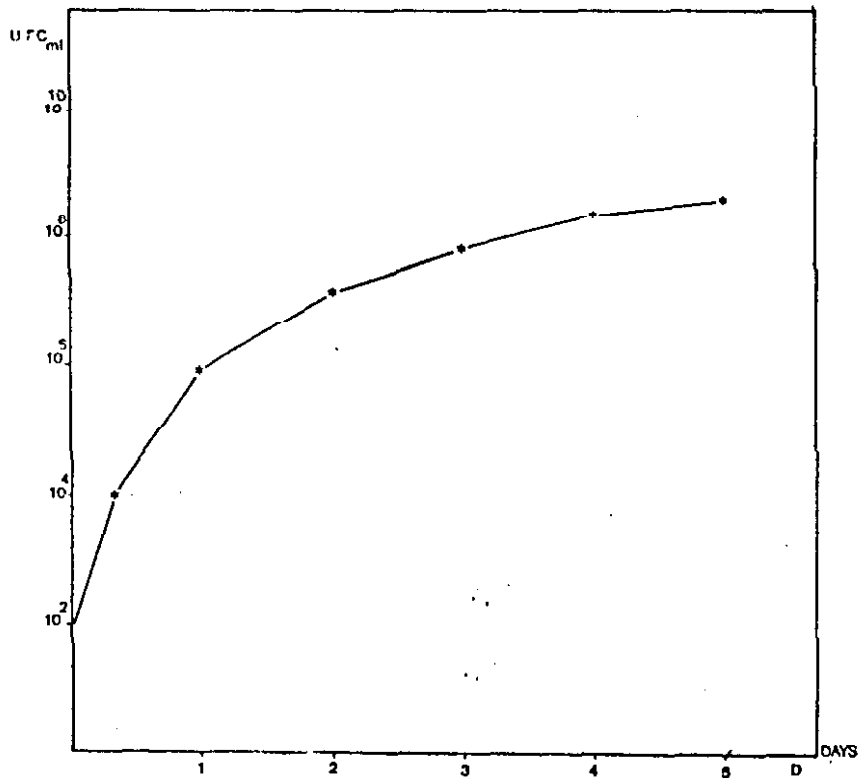


Fig. 1. Crecimiento de *Rhizobium Leguminosarum biovar. Trifolii* en un medio de cultivo standard sin incorporar aminoácidos libres de LBE.

Fig. 1. Growth yield of *Rhizobium Leguminosarum biovar. Trifolii* in a standard culture without addition of a free amino acids complex.

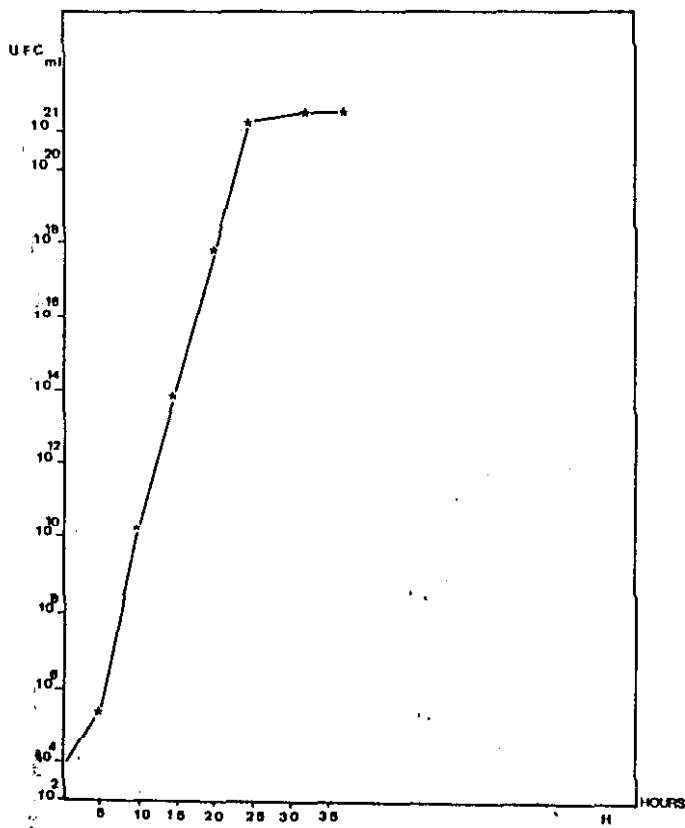


Fig. 2. Crecimiento de *Rhizobium Leguminosarum biovar. Trifolii* en un medio de cultivo standard con adición de aminoácidos libres de LBE.

Fig. 2. Growth yield of *Rhizobium Leguminosarum biovar. Trifolii* in a standard culture with addition of a free amino acids complex.

was rejected in order to avoid multiplication by experimental errors.

The results were entirely unexpected, since, as can be seen from figure 2, the latency phase is virtually nonexistent and is followed by an extremely marked exponential phase, reaching a sudden maximum at about 25 hours, giving a count of 1.1×10^{21} UFC/ml. These results show the enormous importance of the inclusion of free amino acids as stimulants of certain biological processes since, having sugar available in large quantities, it appears clear that Rhizobium does not use them as majority nutrients but rather as modulators of the metabolic processes. This influence can be summarised in the reduction of the generation time and, therefore, in the increase in the growth rate. At present, the points of actuation for this control by amino acids are being sought.

Microorganisms

The complete list of microorganisms results too long and tedious for this paper. Some most important strains for the process are selected in the Table III.

The amino acid complex prepared by Dr. Cebrian in LBE for these culture media was both in crystalline granulated form, of a yellow-ish colour, and in a sterile water solution at 1/50.

The composition confirmed by the Institut Pasteur (1.992) is as follows, in percentage of the amino-acid concentrate:

Ala	8.12	His	0.75	Phe	2.07
Arg	3.96	Hyp	13.21	Pro	16.99
Asn	6.23	Ile	1.32	Ser	0.37
Cys	0.09	Leu	3.02	Trp	0.47
Glu	10.76	Lys	3.87	Tyr	1.79
Gly	23.60	Met	0.94	Val	2.26

Table IV Composition of the amino acide complex

INDUSTRIAL PROCESS

For the industrial process, have been carried out experimentations with four different substrats of plant origin, and whose cellulose, hemicellulose and lignin contents are given in Table V.

Beginning with slant cultures have prepared suspensions and successive re inoculations on different culture media, adequate for each microorganism.

For the pilot plant trials, a fermenter was used with orbital agitation, of 1.5 litres, designed and fine-tuned by us expressly for this experiment. With the content of this fermenter, we sowed the substrat in one of 1500 litres, open-cell type, 1.5 x 1 x 1 m, and this volume was used for the industrial process, at rectangular dimensions of 4 x 3 x 2 m, open..

With this configuration, a 12 cell battery was designed, situated in a fitted room with air supply.

The control of fermentation parameters has been done by a Electronic Control Unit (ECU), specially useful for the rising and control of temperature of the some starters and biocatalists, from 4° C (storage) to 25° C (incorporation to the process).

The problem is to raise the inoculum and biocatalyst temperature from 4° C, the temperature at which they are stored in the coolers, to the 20-25°C level at which they are incorporated into the process, so as to obtain maximum yield; this was done with the use of a programmable relay which controls the flow along the line and the gradual raising of the temperature according to a function which combins a number of variables; the slope of the logarithmic curve of growth of the most important stocks, the concentration of amino acids, the inoculum volume/substrat volume ratio, etc.

The application of the named electronic control unit is very important to control the temperature.

Probes of Al-Cr-Ni for the supervision and control of the fermentation temperature pick up the signals. It was necessary to incorporate analog-digital signal converters in order to introduce these into the E.C.U. (programmable relay) memory. Likewise with the other parameters, pH, oxygen concentration, air supply, redox potential, etc.

The control of the product is verified by chemical and bacteriological analysis, with germ counts by the most probable number method, by

statistical inference and by random sampling pursuant to a specialized statistical method. This methodology was developed by one of the Plant Directors.

On the basis of this configuration, an industrial plant and annexes was designed with capacity for the treatment of 20,000 metric tons of organic substrates (peats or agricultural residues) and to obtain an output of active biomass/biological fertiliser of 12,000 metric tons/annum.

This industrial plant, whose processing chart is shown in figure 3, has been used as a pilot plant since 1981. It is sited in Valencia, very close to the Mediterranean coast.

The last technology application from 1984, with the accumulated experience in the development of this process, have allowed to obtain a high yielding in the active biomass production, at elevated concentrations and with large activity period, which make possible your survival and viability for marketable characteristics as biological fertilizer.

The upshot of all this has been a high yield in the obtaining of active biomass, with a long period of activity, at high concentrations, which makes it viable for marketing as a biological fertilizer.

The incorporation of the biological fertilizer BIORGAN FORTE at doses between 200 and 1200 kg/ha (according to soil type, crop, etc.) gives very significant results which are highly positive in the plant development.

The basic action of biological fertilization is carried out on the organic soil material, in the transformation process which is called "mineralisation". Nevertheless, the micro-organisms do act directly and "per se" on the life of the plant by means of the release of products, such as nutritive substances, growth regulators, aminated acids, vitamins, etc., the result of their own metabolism.

In acting on the organic material, these micro-organisms generate the formation of humus.

Soil without humus is an inert stratum.

Humus has a high capacity for cationic exchange, of the order of 200 milliequivalents per 100 g, in other words, 20 times greater than kaolinite (which has 10 milliequivalents per 100 g) and twice that of montmorillonite with 100 equivalents per 100 g (this clay, thanks to its interlaminary spaces, is the best one for agriculture).

The high humus exchange capacity represents a large benefit for plant nutrition; it seems to be well proven that the cations retained by the humus are more easily absorbed by the radicular system than those fixed by the clay and, in particular, it intervenes in metabolism of phosphor. In addition, the ammonium and potassium of humus is more easily assimilated than that of clay, always through the soil solution, by way of ionic exchanges since the roots only absorb nutritive substances of that liquid.

Apart from the effects on texture and structure, substantial effects are to be noted on the reductions of accumulated quantities of harmful residues from pesticides, herbicides, etc.

A very specific case is the situation set up by the disinfection of soils in glass-houses, which lead to considerable problems of fertility, and in phytosanitary conditions as the result of a feed-back process which is corrected with the addition of the product.

In unirrigated soils, where chemical elements accumulate, addition of the biological fertilizer activates them very favourably.

In highly saline soils, the action of BIORGAN turns out to be very positive, with the recovery of citrus plantations which had been abandoned.

Mention must also be made of the regeneration of artificial fields, with 8 years of life, recovered at production levels of 3-4 years (experiments in areas in the North of Spain).

There has been a proliferation in this country of fowl and pig farms, where feeding has used large quantities of antibiotics, and the droppings from these animals are sold and used in many regions, provoking some highly negative effects. The application of a biological fertilizer of the type described here has been shown to cancel the negative effects of antibiotics on soil micro-organisms.

ALTERATION OF SPOTS ON TOBACCO: EXPERIMENTS WITH AND RESULTS OF THE PRODUCT
"BIORGAN"

This alteration appeared during the 1984 season in the Spanish province of Cáceres on Virginia type tobacco plants; at the moment of maturity, brown tonings were noted, not very clearly defined, on the leaves, and accompanied by a curling of the edges of the leaves. These marks appeared uniformly, beginning from the tip.

During the drying process, the leaves darkened further and took on a leaden appearance, acquiring neither colour nor aroma, nor becoming humid, so that when dried, they are brittle and of no commercial value.

Work began in 1984, with the direct involvement of the Technical Engineer Félix Teixeira, Head of Crops of the National Tobacco Crop and Fermentation Service, and Professor Carlos de Liñán, Professor of Phytopathology of the "ETSIA" in the Polytechnic University of Madrid.

In the first year, as a result of direct and laboratory observation of affected plants, the idea of parasitic or pathogenous origin was rejected. In the light of the leaf and soil analyses made (clear excess of phosphor), the conclusion was reached that the condition must be due to a nutritional imbalance.

The products tried as possible control, the effects of which continue to be positive, include the use of 800 kg of BIORGAN FORTE/ha, and an increase by 80-100 units of nitrogen over those normally used.

The use of nitrogen appears positive until the harvest of the 2nd:4th layer; at all events, more or less serious symptoms of the alteration are normally observed. The plants die following the harvest.

With the use of BIORGAN, there is a more continuous effect, without the appearance of symptoms. Once the harvest is over, the plants are treated, and there are no serious symptoms noted of the alteration.

Uprooted plants treated with phosphor (P), nitrogen (N) and

Dipotassium phosphate.....	0.5 g	Monopotassium phosphate.....	0.4 g
Magnesium sulphate.....	0.2	Dipotassium phosphate.....	0.5
Yeast extract.....	0.4	Magnesium sulphate.....	1.4
Manitol.....	10.0	Sodium chloride.....	0.1
Tryptophan.....	0.2	Manitol.....	10.0
Water.....	1 L.	Tryptophan.....	0.2
		Aminoacid complex.....	2.5
		Ferrous sulphate.....	0.1
		Winogradsky's solution.....	10 ml
		Water.....	990 ml.

Table 1: Culture medium M-1.

Table II: Culture medium M-2.

BACTERIA:

- Sulfobacteriaceae: Desulfovibrio.
- Ferrobacterias: Thiobacteriaceae: Thiobacillus.
- Nitrogen fixers: Nitrobacter, Nitrosomonas.
- Facultative anaerobes: Klebsiella, Enterobacter.
- Bacillus polymyxa, B. cereus, B. macera ..
- Lactobacillus plantarum, L. bulgaricus.
- Cellulolytic bacteria.
- Nitrogen fixers, free life: Azotobacter, Beijerinckia.
- Symbiotic fixers: Rhizobium.
- Actinomycetaceae: Arthrobacter, Nocardia, Streptomyces.

FUNGI:

Mucor, Aspergillus.

ALGAE:

Nostoc, Anabaena, Volvox, Navicula.

Table III: Some of microorganisms used in the process.

Ala 8.12; Arg 3.96; Asn 6.23; Cys 0.09; Glu 10.76; Gly 23.60;
 His 0.75; Hyp 13.31; Ile 1.32; Leu 3.02; Lys 3.87; Met 0.94;
 Phe 2.07; Pro 16.99; Ser 0.37; Trp 0.47; Tyr 1.79; Val 2.26.

Table IV: Composition of the free aminoacid complex.

	Substrat I*	Substrat II	Substrat III	Substrat IV
% Cellulose	-	37.0	41.0	46.0
% Hemicellulose	12.0	42.0	32.0	25.0
% Lignin	35.0	17.0	21.2	24.0
Relation C/N	15.0	28.0	33.0	38.0

Table V: Composition of substrats incorporated in the process. Percentage of total dry material. (*) Fermented cereal straw.

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DIAGRAM PROCESS MANUFACTURING ACTIVE BIOMASS AS BIOLOGICAL FERTILIZER (BIORGAN 220-GRANULATED)

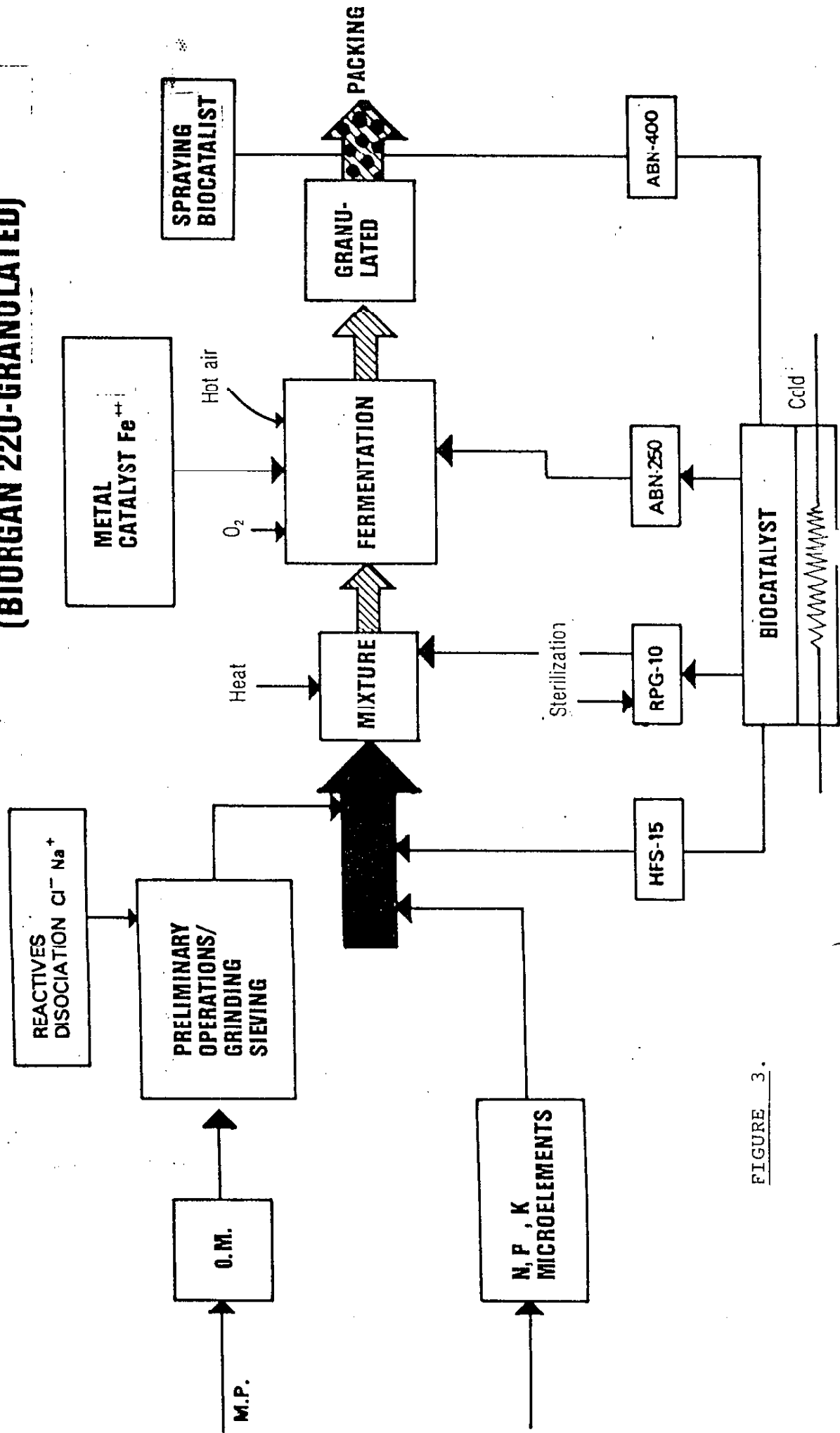


FIGURE 3.

ACTUACION REGENERATIVA EN SUELOS DE LA BIOMASA / ABONO BIOLÓGICO "AMINORGAN / BIORGAN" EN FUNCION DE SU COMPOSICION

INAGROSA
Velazquez. 31
MADRID 28001
Dpto. Técnico

COMPOSICION QUIMICA % S.M.S S/ Register 6323	INTENSIDADES DINAMICAS (ID)			COMPOSICION MICROBIOLOGICA IMP: n°/gcf Recuento a los 90 días	ID		
	ED	AB	AG		ED	AB	AG
Materia Orgánica 40%	++	++	++	HONGOS 250.000	++	+++	++
N 3%	0	++	+	ALGAS 80.000	++	++	++
P ₂ O ₅ 0.5%	0	++	+	CIANOFICEAS (G. Nostoc) 10.000	++	++	++
K ₂ O 0.5%	0	++	+	ACTINOMICETOS 120.000	++	+++	++
MICROELEMENTOS	0	++	+	BACTERIA VIVIFICABLE 1x10 ¹¹	++	++	++
Mg 0.05 %				ACTINOMICETES AZOTOBACTERIA RIZOSIUM AGROBACTERIA OTRAS	+++	+++	+++
Fe 0.054 %							
Mn 0.013 %							
C ⁺⁺							
CINa	-X	O/E	SSS				
Na		SSS	SSS				

COMPOSICION % sobre precio venta al publico	ID	COMPOSICION % EN PESO	ESTRUCTURA VALOR ANADIDO SOBRE PRECIO VENTA AL PUBLICO
M.O 10	++	1.0	MAND DE OERA 10.5
NPK 11	0	NPK	ENERGIA Y OTROS 1.5
MICROELEMENTOS 4	0	MICROELEMENTOS	COSTES EXTERNOS DIRECCION Y MANTENIMIENTO 6.0
REACTIVOS QUIMICOS 4	++	REACTIVOS	BENEFICIO BRUTO PARA INAGROSA 80.0
OTRAS MATERIAS PRIMAS 3	+++	BIOCATALIZADORES Y OTRAS MATERIAS	BENEFICIO BRUTO DISTRIBUCION, TRANSPORTE Y C. FINANCIACION 82.0
BIOCATALIZADORES 32	+++		-----
VALOR ANADIDO 35	+++		100.0

100			

ID = Intensidad Dinámica de Actuación

ED = Edafológica
AB = Agrobiológica
AG = Agronómica

+++ MUY INTENSA Y POSITIVA.
++ NORMAL Y POSITIVA.
+ REDUCIDA Y POSITIVA.
0 CERO.
O/E CERO EN SUELOS EQUILIBRADOS.
SSS MUY INTENSA Y POSITIVA EN SUELOS MUY SALINOS.
SS NORMAL Y POSITIVA EN SUELOS LIGERAMENTE SALINOS.
-X INTENSIDAD NEGATIVA, REDUCIDA EN SUELOS MUY POREOS Y CON ESCASO DRENAJE.

(1) IMP (número más probable.)

(2) SPP (Bicc):
Cepas seleccionadas por INAGROSA y activadas.

(3) PARA UNA OCUPACION DEL 60% DE LA CAPACIDAD DE PRODUCCION.

BIOTECNOLOGIA APLICADA A LA AGRICULTURA

ESQUEMA DE DISEÑO DEL PROCESO DE FABRICACION DEL FERTILIZANTE BIOLÓGICO "BIORGAN" PARA CADA TIPO DE SUELOS CON EMPLEO DE BIOREACTORES Y FACTORES DE CRECIMIENTO FACE (CELL GROWTH FACTOR).

