AGRONOMIC APPLICATIONS FOR CYANOBACTERIA AND MICROALGAE
AN INTERNATIONAL EVALUATION

DOCTORAL THESIS
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I. INTRODUCTION:

The purpose of this research work is to investigate and evaluate practical application for microbiological concepts as solutions for soil and cropping related problems in global agriculture.

The motivation for this work is expressed by a recent quotation from an Associated Press – Farm Writer, “Much of the world’s farmland is in such poor condition that farmers will have to find better ways to grow crops or else their production won’t keep pace with the growing population”.¹

Soils world wide, as a negative by-product of modern synthetic chemical and machined systems of agriculture, are suffering from soil compaction, poor water holding capacity and declining capacity to carry a crop. This is frequently associated with declining quantities of important organic matter components within the soil chemistry. It is the intent of this research to study the concept of using microbiological agents to improve the physical properties of soils and associated with these improvements determine crop response in the form of yield.

Cyanobacteria and chlorophytic microalgae are related and very ancient microorganisms. Cyanobacteria are thought to be the first terrestrial organism to grow on earth. These microbes are commonly given credit for the origin of the earth’s crust. By learning to grow outside of the ocean environment, in the cracks and crevices of the stone

which originally covered the earth’s surface, this organism began to deposit organic materials which interacted with the mineral content of the stone, thus began the earth’s top soil. Cyanobacteria and also microalgae have long been recognized as contributors to the origin as well as the ongoing maintenance of the earth’s top soil.

In recent work the use of cyanobacteria and microalgae have been documented in the literature as agronomically important in a flooded rice field. Much research has been conducted on this topic. To date, little work has been done to document the value of these same microbes in the non-flooded crop production system. This study focuses on testing the concept of applying cyanobacteria and microalgae, as microbiological soil inoculants, within the agronomic context of non-flooded on-farm cropping systems (i.e. either totally dry-land or with minor irrigation). The goal of the study is to determine soil physical property changes and plant responses (primarily in the form of yield data) associated with treatment.

II. LITERATURE REVIEW AND RELATED DISCUSSION:

Introduction: The positive effect of favorable soil structure and negative effects of soil compaction on crop growth and/or yield are repeatedly illustrated in the literature. Research more frequently addressed the soil structure effects on crop growth and yield in the 1940’s, 1950’s and early 1960’s. The effects of soil structure on growth
and yield were demonstrated for crops ranging from onions (Rynasiewicz, 1945) to field corn (Page and Willard, 1947).

Soil structural conditions interact with the above ground environment to produce the soil environmental conditions that influence crop growth. A variety of research efforts have compared crop growth and/or yield between plot areas having different soil structural conditions. These efforts consistently illustrate a positive crop yield (or growth) response to what would be considered the most favorable soil condition. Grain yield increases of from 23% (Barber, 1959) to 39% (Swanson and Jacobson, 1956) are observed as one compares yield from the “undesirable” soil condition to the “most desirable” condition. Onion yields increased 210% on the favorable compared to the unfavorable condition (Rynasiewicz, 1945).

The literature on this subject suggest a lack of research continuity over the years in the effort to relate soil structural conditions, soil tilth, soil aggregation or degree of compaction to yield. Even with this, few agriculturalists would argue about the value that structure has in crop production.

**Soil Aggregation:** Soil aggregates are structural units composed of both mineral and organic matter. Aggregates can be, and generally are, orders of magnitude larger than the primary particles which comprise them. By increasing the size of the structural units above the size of the primary particles, various soil physical properties are improved.
Organic matter additions to soil have long been known to improve structural conditions. A variety of studies have illustrated the correlation between soil aggregation and organic matter additions to soil (Harris et al., 1966; Rennie et al., 1954; Tisdall and Oades, 1982; McHenry and Russell, 1944).

Organic material, such as plant residues, generally has a very minor effect on aggregation until these materials are “acted upon” by microbes (Lynch, 1981; Myers and McCalla, 1941; McHenry and Russell, 1944). Selected microbes degrade the more complex materials, and produce from these sources microbial gums which have a direct effect on aggregation (McHenry and Russell, 1944); Tisdall and Oades, 1982). The microbial gums frequently contain long-chain organic molecules, essentially linked sugar molecules, that are referred to as polysaccharides. The polysaccharides produced may in turn be degraded by other organisms reducing the quantity of polysaccharides available for aggregate development, even though the degrading organism may produce polysaccharide itself. Thus microbes are very important in polysaccharide degradation as well as in its production.

Polysaccharides can be very effective soil binding agents and were identified as the most important single factor affecting aggregation of four soils in a study by Chesters et. al., (1957).

Polysaccharides are organic molecular chains which have attached to the main carbon chain various chemical configurations or branches. The
chemical characteristics of these branches determines to a large extent how well the polysaccharide will bond to the soil particles. Selected favorable characteristics of polysaccharides are responsible for the positive effects which most polysaccharides have on soil aggregation. Clapp and Emerson (1972) and Parfitt (1972) summarized three basic polymer properties and chemical actions which allow bonding to mineral surfaces: 1) Uncharged polymers which have hydroxyl (-OH) functional groups may be adsorbed to clay surfaces by hydrogen bonding. 2) Negatively charged polymers or portion of polymers which contain carboxyl functional groups may develop bonds with positively charged cations bonded to the negatively charged clay surface. The cation works as a “bridge” linking the polymer with the clay particle. Uronic acid segments, also negatively charged, found on selected polysaccharides may also bond to the cation bridge. 3) Positively charged sites on the polymer, those containing amino functional groups, may bond directly to the negatively charged clay surfaces. These reactions are similar to the cation exchange reactions which occur in soil (i.e., they are not dependent on ion bridging between the polymer and mineral surface). The diversity of bonding mechanisms allows polysaccharides to become “attached” to mineral surfaces under a variety of circumstances.

The functional groups occurring on the polysaccharide molecules as well as the physical characteristics of this molecule was emphasized by Martin (1971) as he reviewed previous work of Greenland (1965), Harris
et al., (1966), Martin et al., (1955) and Ruehrwein and Ward (1952).

Martin (1971) concluded that the effectiveness of polysaccharide in binding soil particles together was attributed to: 1) their length and linear structure which allows them to bridge space between particles; 2) their flexible nature which allows a large number of points in close contact so that Van der Waals’s forces can be effective; 3) the large number of hydroxyl groups which may be involved in hydrogen bonding; and 4) acid groups, primarily carboxyl, which allows ionic bonding through di- and trivalent cations to ion exchange sites on clays or of anion adsorption to positive charge sites on clay edges.

Soil texture determines to a large extent the influence of polysaccharide on soil structure. Clay particles, due to their surface charge characteristics, tend to enhance the polysaccharide bonding activity. Sand particles tend to reduce the effects of polysaccharide on aggregation (Edwards and Bremmer, 1967). Even though sand particles may weaken aggregates (Edwards and Bremmer, 1967); Forster (1979) indicated microbial production of a polysaccharide-like material resulted in some aggregation of sands.

Because polysaccharides appear to constitute a nearly ideal molecular form for enhancing aggregation of soils containing clay, increasing soil polysaccharide content might be expected to improve structural conditions of most clay containing soils, especially those with lower organic matter content. Martin (1971) in reviewing work by Gupta and
Sowden (1965; Mehta et al., (1961); Oades (1966); Parsons and Tinsley (1961) and Swincer et al., (1968a, 1968b, 1969) indicate polysaccharides may account for 5 to 30 percent of the soil organic matter content. For a soil having 6% organic matter in the plow layer, this could mean from 6,000 to 36,000 lbs. of polysaccharide per acre (6,800kg to 40,900kg per hectare) in the top six inches (16 cm) of soil. Rennie et al., (1954) indicated as little as .02g of polysaccharide synthesized by *Agrobacterium radiobacter* added to 100g of two silt loams increased aggregation considerably for both soils. This level of polysaccharide addition is equivalent to roughly 400 lbs. polysaccharide added to one acre of soil (454kg/ha) and distributed uniformly to a 6 inch (15 cm) depth. Greenland et al., (1962) showed that organic molecules including polysaccharides were the primary aggregate stabilizers in continuously cropped soils or young pastures. Thus, polysaccharide additions to soil would most likely play a prominent role in soil structure when soils are intensively cropped and/or frequently disturbed by physical forces such as tillage. Soil structural conditions (aggregation and/or compaction) have a direct effect on the soil environment, the soil environment being of critical importance to the performance of a growing crop.

**Soil Environment:** The soil environmental factors to which plant roots respond include: water, aeration, nutrition, temperature, toxicities, and soil strength. The physical properties of the soil solids interact with the above ground environment to determine the soil environmental
condition. It is because of this interaction that the physical arrangement of soil solids, or soil conditions, can have a major impact on plant growth and crop production.

Water considerations are controlled by four major activities: infiltration, drainage, evaporation and transpiration. Many factors influence the dynamics of water in a soil, but one major influence is the physical condition of the soil.

Drainage of a soil is increased with physical aggregation of the soil particles. Increased aggregation of a soil profile allows the pores between aggregates to increase, thus allowing for better drainage (more saturated conductivity) and better corresponding aeration (Grable, 1971). At the same time increased aggregation will also increase water holding pore space inside aggregates, which produces better retention of water under more dry soil moisture conditions (less unsaturated conductivity) for later plant use (Warkentin, 1971).

Water infiltration during rainstorms is influenced by the infiltration capacity of the soil. Infiltration capacity of the soil tends to be much greater for soils which have relatively large open pores on the soil surface than for those soils which have smaller pores (Kemper and Miller, 1974). Stable soil aggregates which reduce soil dispersion during a rainstorm or overhead irrigation practice tend to promote high infiltration rates (Hartmann et al., 1981) and reduced run-off. Leving (1959) concluded that the decrease in a soil’s infiltration capacity during the course of a
rainstorm was due to compaction and sealing of the surface layer. Water must infiltrate before it can be deposited in the soil moisture bank. Shrader and Murcock (1970) indicated that for Western Iowa, USA, each additional inch (2.5 cm) of stored soil water would result in, on the average, about 10 bushels per acre additional corn yields.

Water lost from an agricultural field due to evaporation is unavailable for crop production. Water lost through evaporation has a negative effect on crop yield and can account for as much as 50% of the total water loss from row crop fields in the Midwest, USA (Peters, 1960). The role of soil structural conditions in evaporation losses can be quite significant (Lemon, 1956; Papendick et al., 1973; Willis and Bond, 1971; Holmes et al., 1960; Allmaras, 1967). A well structured soil condition will likely have a lower unsaturated hydraulic conductivity than the poorly structured condition which would slow the water flow to the soil surface during times of low soil moisture, thus decreased evaporation, which in turn favors water storage.

The reduction in pore space and aeration which accompanies soil structure degradation has been blamed by various researchers for crop growth and/or yield reductions (Baver and Farnsworth, 1941, Lawton, 1945; Quastel, 1952; Gill and Miller, 1956; Hagin 1952). Under most conditions in which there is less than 10% air filled pore space (10% by volume of the soil is filled with air), gas diffusivity approaches and many times equals zero (Grable, 1971), producing a so called soil anaerobic
condition. When plant roots are exposed to anaerobic conditions for time periods as short as 24 hours, permanent root growth reductions may result (Erickson and Van Doren, 1961).

Nitrogen is a plant nutrient which is water soluble. Fertilizer applied nitrogen use efficiency is reduced whenever nitrogen in the plant available form of nitrate is leached below the rooting zone. Since water movement during infiltration is dominantly through the large pores, i.e., around the outside of aggregates, it is believed nitrate retained inside the aggregate will be less susceptible to leaching than if poor aggregation existed and water moves around all particles. This is supported by work of Agrawal e. al. (1984) and Saglam (1975). Wheat yields increased with increasing size aggregates in Agrawal’s study. Further, Argawal’s study showed that the yield differences, between soils with aggregate size differences, increase as nitrogen application rate increased. Soil with the coarser aggregates had the higher yields. Nitrogen uptake is a complex part of the overall soil/plant relationship. While these studies do not prove structural improvement will always improve nitrogen uptake efficiency, they do suggest the possibility.

Compaction increases soil strength and/or mechanical resistance to root growth and seedling emergence. Compaction effects are frequently confounded with aeration and sometimes water stresses. Soil structural conditions can have major impacts on soil mechanical resistance to root growth.
Roots forced to grow through soils with high mechanical impedance (high resistance to root elongation) become shorter, thicker and have more densely packed cortical cells (Schumacher and Smucker, 1981). The volume of soil which can be explored for water and nutrients is decreased, reducing the quantities of both water and nutrients which might be taken up by the plant. Taylor (1971) diagrammed the relationship between cotton seed yield and penetrometer resistance obtained from data published by Carter et al., (1965) and Carter and Tavernetti (1968). Penetrometer resistance was that of a high strength pan immediately below the cotton row on an irrigated sandy soil. Cotton seed yield decreased linearly from approximately 3600 kg/ha to 1200 kg/ha as penetrometer resistance of the pan increased from 5 bars to 30 bars.

Soil crusting or sealing may effect emergence of crop seedlings (Kemper and Miller, 1974; Miller and Gifford, 1974; Bennet et al., 1964; Taylor, 1971; Hanks and Thorp, 1957). Soils high in organic matter (Vehara and Jones, 1974) tend to resist crust development while those high in silt and low in organic matter tend to more easily develop strong crust (Kemper and Miller, 1974). Strivers and Swearingin (1980) compared the effect of different skip lengths (row length with missing soybean plants) alternated every other row to a complete stand as they affect soybean yields. As skip length increased from 0.39 m to 0.91 m yields decreased. While the dicotyledonous crops (soybeans) are most
sensitive to crusted soil conditions with respect to emergence, they do
tend to show some capacity to adjust to population fluctuations compared
to other crops such as corn. For monocotyledonous corn the average
yield of individual plants within a given population, has a more
predictable linear relationship with populations (Duncan, 1958).

It can be stated that compaction seldom favors the economics of crop
production and generally works against it. Gill (1971) speculated that the
annual loss in crop value due to compaction in the United States may
exceed $1.0 billion annually. This value does not take into account
additional potential production cost such as those for increased power
and machinery requirements, energy consumption, and irrigation, as well
as extra management cost.

Managed Microbial Contribution To Soil Properties and Crop
Response: Several microalgae of the Chlorophyceae and the
Cyanobacteria microbial groups have selected characteristics which
make them a potential management tool for crop agriculture. (Metting
and Rayburn, 1983). These organisms are high polysaccharide producers
(Kroen, 1984; Kroen and Rayburn, 1984) which make them appear
favorable for improving soil structural conditions. These
microorganisms can survive and flourish in a wide range of
environmental conditions ranging from barren rock (Booth, 1941) to
fertile moist substrates (Alexander, 1967). Selected individuals can survive prolonged, intense freezing (Hansen, 1963) while others can survive hot dry conditions (Trainer, 1970).

For a managed microbial polysaccharide contribution to soils, utilizing selected chlorophytic microalgae and cyanobacteria, the steps are: 1) apply vegetative or dormant resting cells to the soil surface; 2) permit the population of cells to become established and reproduce or duplicate many times to reach a maximum; and 3) cause the cells to produce polysaccharide in abundance for improvement of soil structural conditions. Kroen and Rayburn (1984) have demonstrated the growth and polysaccharide production cycle of the green microalgae, *Chlamydomonas mexicana*, grown in liquid medium. The population starts at a low number, grows exponentially, and reaches a maximum population number. The polysaccharide production and cell division may not occur simultaneously. Polysaccharide production lagged behind the growth curve in the studies of Kroen (1984) and Kroen and Rayburn (1984) for this green alga. Polysaccharide production did not peak until the stationary growth phase was reached. Kroen and Rayburn (1984) enhanced polysaccharide production by inducing the stationary growth phase through nutrient depletion in the growth media. Kroen (1984) also observed a similar relationship between cessation of cell division and polysaccharide production.
Microalgae and cyanobacteria may colonize the soil surface or that area just below the surface, where soil pore sizes may physically allow the microbes to exist slightly beneath the surface. Onofoik and Singer (1984) indicate the average pore diameter in the surface crust of three separate soils ranged from approximately 5.0 microns to 7.5 microns. With uncrusted conditions average pore diameter were estimated at about 12 microns. The microbes in question average 5.0 microns in diameter. Metting and Rayburn (1979) found on an eastern Washington (USA) silt loam soil the algal populations at the surface at 124.4 x 10³ and at a depth of five centimeters the count was 22.0 x 10³. Several species of algae and cyanobacteria constitute an initial stage in plant succession by the formation of a microbial crust over hundreds of acres in the south central USA (Booth, 1941). Fletcher and Martin (1948) found an algal/cyanobacteria crust in the desert near Tucson, Arizona, USA, which grew well in desert conditions, which microbial complex contributed an increase in soil organic carbon as high as 300 percent and organic nitrogen by 400 percent, while the author noted decreased soil erosion, improved infiltration and improved establishment of plant seedlings.

The most active soil binding substances synthesized by effective microbial species are polysaccharides and test by Martin (1971) showed that microbial polysaccharides in concentrations of 0.02 – 0.2% exert a marked binding action on soil particles. Bailey et al. (1973) observed that
algae and cyanobacteria are important in stabilizing and improving the physical properties of soil as a medium for plant growth. Metting and Rayburn (1983) observed that when *Chlamydomonas* microalgae was inoculated repeatedly on selected Washington, USA, soils, carbohydrate concentration was higher in the surface 1cm and the upper 30cm over controls, water retention was greater in the treated fields and both wet and dry aggregate stability were greater under treatment in the three selected soils. In a comparison using extracted *Chlamydomonas* polymer and the commercial polyvinyl alcohol Krillium®, the stability of soil aggregates using microalgal polysaccharides were significantly greater than either the controls or the aggregates stabilized with the Krillium® (Metting and Rayburn, 1983). On a microscopic level, the mechanism for improved soil structure by addition of microalgal/cyanobacterial polysaccharide has been photographed by electron microscopy as fibers and gels of polysaccharide that are in crevices and between stacks of clay platelets that bind them together, even forming box-like structures which resist dispersion or collapse (Foster, 1981). Polysaccharides may fill spaces too small for bacteria to get to them, thus reducing microbial decomposition, enmeshing large volumes of soil, further suggesting why small quantities (0.02 – 0.2%) of added polysaccharide markedly stabilizes clay aggregates (Foster, 1981).

Bailey et. al. (1973) measured the capacity of three microbes to aggregate clay particles. The test involved the chloropyhte *Chlorella*...
pyrenoidosa and the cyanobacteria *Nostoc commune* and *Oscillatoria prolifera*. The soil was a Peoria loess soil and the test incubation period was six weeks. The microbial treatments produced water-stable aggregates in the 1000 – 2000 micron diameter range for the cyanophytes and the 500 – 1000 micron range for the chlorophyte, while the control soil showed no aggregates above 295 micron.

Trainor (1970) observed that microalgae and cyanobacteria survive over time in soils. Connecticut, USA, soil samples were collected and analyzed in 1958. After ten years the samples were again evaluated. Out of thirty different algal and cyanobacteria species originally isolated, eleven survived 11 years in dry storage.

Yield increases associated with the inoculation of paddy rice with cyanobacteria has been broadly reported in the literature. Relwani and Manna (1964) found that rice grain and straw yields in plots fertilized with the cyanobacteria *Nostoc* and *Tolypothrix*, and lime, superphosphate and sodium-molybdate, had greater yields than for plots fertilized with urea plus the other three chemicals. Similar reports of improved yields due to applications of cyanobacteria in rice fields include those of Jha et al. (1965), in which an increase of 36% was attributed to *Tolypothrix*. Rice yield increases associated with field inoculation with cyanobacteria have been attributed to the capacity of cyanobacteria to fix and release into the growing medium quantities of plant available nitrogen. Investigators have reported cyanobacteria fixation of nitrogen in field
conditions from China (Ley, 1959), Vietnam (Mishustin, 1964), the Philippines (Watanabe et al., 1977), Japan (Watanabe, 1951), Egypt (Nawawy et al., 1958), the Soviet Union (Perminova, 1964; Shtina, 1965), and India (Singh, 1961; Venkataraman, 1975). In addition to the influence on crop growth resulting from fixation of nitrogen, cyanobacteria are known to positively alter productivity by other means. Included are chelation of inorganic plant nutrients in the soil solution by extracellular organic compounds “leaked” from cyanobacteria (Okuda and Ymaguchi, 1960), production of growth-promoting vitamin and hormone-like substances (Venkataraman and Neelakantan, 1967), solubilization of inorganic phosphorous compounds (Venkataraman, 1975), and improvement of structure via aggregation of soil particles (Roger and Kulasooriya, 1980).
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III. METHODOLOGY:

This research study involves field experimentation on multiple continents. The international sites selected involve both northern and southern hemisphere locations. The fields utilized in the study were generally involved in what is called dry-land or light-irrigation agriculture, as opposed to, for example, flooded field conditions common to rice farming. Soil types for this work fall into a range between what is generally called a clay loam to a silt loam textural composition. As much as possible the field conditions under which the trials were conducted were organized to represent actual farming conditions. In most cases the trials were in actual fields owned by a local grower, with a multi-year history of continuous farming.

The common plot design for the experiments involved the randomized complete block method. In most cases, plots were organized as pairs and pairs treated as blocks (or replicates).

Yield evaluations were determined using standard methods which utilize hand count of a fraction of the plot, duplicating the effort as multiple subsample measures per plot and finally creating a mean for the plot from the subsample data. Means were compared to determine significance. Metric and English measures were reported, depending on location (i.e. results in U.S.A. used English and outside U.S.A. used metric).
At some field sites laboratory measures were taken for soil samples taken from treated and control plots. Wet aggregate stability test were conducted to determine the stability of the aggregate profile under the forces of agitated water. Modulus of rupture evaluations were generated to determine soil strength factors. Means were compared to determine significance.

Field soil compaction measures were taken utilizing a penetrometer. Multiple subsample measures were taken per plot and a mean for the plot was determined from the subsample measures. Means were compared to determine significance.

The treatment reported on herein is a constant and standardized composition of photosynthetic microbes composed of the green microalga genus *Chlamydomonas*, *Chlorella* and the cyanobacteria genus *Tolypothrix* and *Anabaena*. The purpose of the composition is to broadcast a seeding (or inoculation) of viable dormant photosynthesizing microbes at the rate of $9.1 \times 10^9$ cells per acre to the soil surface, thereby establishing an artificial advantage for this inoculation of selected microbes, and allowing this selected microbial population to grow for three to five weeks, without significant top-soil disturbance. The artificially induced large population of selected microbes are known producers of biopolymers or microbial polysaccharides, an organic soil-aggregating agent. This seeding practice may be considered a method of
establishing a soil improvement microbial green manure crop, either before a crop is planted or on an intercropped basis.

IV. RESULTS:

In this section the results of multiple international field experiments will be presented. The countries include the U.S.A., Japan and Australia. Each test is presented as an independent case study. All studies were organized, designed, supervised and evaluated by this author. The test sites were on private lands.

Case One: U.S.A.

Introduction: In this test case two types of soil test were chosen as indicators of soil crust strength and durability of soil aggregates. The two tests were modulus of rupture and wet aggregate stability. The modulus of rupture is a well-established test for determining the strength of a briquette of soil made in the laboratory, which models a severe field soil crust. The wet aggregate stability test reveals the durability of soil aggregates when exposed to slaking and agitation in water. This later test method was chosen to ascertain whether the soil aggregate crumb structure caused by the treatment would withstand the stresses of water saturation and agitation.
Method: The treatment in this test was the microbial composition described under Methodology above. The microbial composition, in a dry base of kalonite clay, was diluted and sprayed onto the soil. The site was a dry-land (non-irrigated) farming operation located in Jefferson County, Iowa, U.S.A. The crop history in this field was 1982 and 1983 corn, 1978 – 1981 a mixture of alfalfa and orchard grass. The site was level. The site was subdivided into twenty 10 x 50 feet test plots with intervening 10 feet buffer zones seeded with rye to prevent rain induced lateral migration of the microbial composition from treated plots to neighboring (alternating) control plots. Plots were selected for textural similarity. Textures were analyzed and plots selected to minimize the possibility of data variability produced by texture differenced between plots. In this test the plots 2 and 5 were selected. The master treated plot, Plot 2, texture analysis was 30% sand, 55% silt and 15% clay. For the master control plot, Plot 5, texture analysis was 30% sand, 53% silt and 17% clay. The soil was rototilled in October 1984 and left bare except for weeds. No fertilizers or chemicals were applied. The microbial composition was applied by hand sprayer in November 1984 and again in March 1985 at the per acre rate presented under Methodology above mixed with 20 gallons of water.

Bulk soil samples were assembled from one inch deep trowel scoops taken randomly over each plot on May 23, 1985. In the laboratory, the samples were spread and air dried for 72 hours and then gently crushed
with a rubber mallet. The soil was screened by hand in 8 inch diameter brass wire sieves into two types of bulk samples: (1) all material passing the 2.00 mm sieve and (2) material passing the 2.00 mm but retained on the 1.00 mm sieve. The samples were stored in closed polyethylene buckets.

For the modulus of rupture test the standard method as described by Reeve (1965) was used. A briquette loading device was custom made from hardwood with loading by trickling water. The briquette molds were from Soil Moisture Equipment Corporation, California, U.S.A. Material passing the 2.00 mm sieve and reasonably free of vegetable trash was passed through a plastic funnel into the molds, which rested on filter paper on top of aluminum screen fixed to wooden screen frames. Because most of the soils were fairly well aggregated, it was found necessary to add compaction to the standard method at this stage to produce briquettes that could be handled and tested without crumbling. A pine wood block was placed on the planed soil surface and 11.4 lb. weight gently applied for a few seconds. The average pressure was 3.3 psi. More soil was sprinkled on the compressed surface, planed off and the same compaction repeated. Eight briquettes were manufactured per screen rack, which was wetted from below to saturation and soaked for one hour before drying at 50 degrees F for 36 hours. The briquettes were broken as simple beams in the standard way, their dimensions measured and the
modulus of rupture calculated from the formula for fiber stress in beams, as follows:

\[ S = 3 \frac{F}{L} \frac{1}{2} B D^2 \text{ dynes/cm}^2, \text{ where} \]

- \( F \) = force at rupture
- \( L \) = length of beam between supports
- \( B \) = width of briquette at rupture surface
- \( D \) = height of briquette at rupture surface

For the wet aggregate stability test, the method used closely followed Kemper (1965). Approximately 50 g. of aggregates retained on the 1.00 mm screen were weighed (“initial aggregates and sand”) and wet sieved in carbon filtered tap water for 7 minutes at 30 cycles/min. with a rise of 125 mm per cycle. The screen was a No. 60 (0.25 mm) Soiltest 8 inch diameter sieve. A No. 18 (2.00 mm) screen was placed above it and a No. 10 (1.00 mm) was placed below it to channel the water and keep small aggregates from escaping over the sides, but otherwise the flow of water was unrestricted. The aggregates were immersed rapidly, screened, washed into a drying dish, dried at 100 degrees C overnight and weighed to yield “water stable aggregates and sand”. The weight of sand in the sample was determined by hand washing the dried sample on the same No. 60 screen in a dispersing solution (5g/l sodium metaphosphate). Sand retained on the screen was washed into the drying dish, dried and weighed for subtraction from both water stable aggregates and initial aggregates. Percent water stable aggregates was defined as: \( \% \text{ water} \)
stable aggregates = (wt. of water stable aggregates divided by wt. of initial aggregates) x 100.

The plots were probed in the field in May 1985 using a Soiltest Proving Ring Cone Penetrometer. This device uses a 5 inch diameter steel proving ring with 0.0001 inch dial gage to measure the force required to push a 2 inch long conical steel point into the soil. The point angle is 30 degrees and the area of the cone’s top is one square inch. Twenty readings were made to the depth of the steel point in both control and treated master plots. At the time of testing the moisture content was slightly less than field capacity.

A further field test was conducted on the plots. This was a soybean emergence test to determine a crop response to the soil treatment. A pair of 10 \` x 50 feet plots (plots 15 and 16) at the same site were rototilled and twenty-four planter boxes, four-feet square, were built of 1 x 6 inch lumber and set into the soil. The textural analysis of plots 15 and 16 were identical at 16% sand, 64% silt and 20% clay. Plot 16 was treated with the microbial composition in November 1984 and again in March 1985. A crust was formed by applying one inch of water. Subsequent alternating rain and sun formed a ½ inch crust. Twelve soybean seeds per box were then planted 12 inches apart, at a measured depth of 1.5 inches with minimum disruption of the crust, which was reformed by hand watering. One hundred forty-four seeds each of two varieties were planted. The varieties were Stine 3560 with a high emergence rating of
“1” and Williams 82 with a low emergence rating of “5” on a scale of one to five. The emerging plants were determined several times per week.

**Results:** The three physical properties tested showed significant differences between treated and control plots, see Table 1. The modulus of rupture of the treated soil was significantly lower than the control. The field penetrometer test showed significantly less surface compaction for the treated soil compared to the control. The treated soils produced a higher percent of water stable aggregates.

The emergence study agreed with the physical property test. Table 2 contains the results at 21 days and shows the greater relative benefit to the weaker emerging seed (Williams 82). The higher emergence of the treated soil became significant after the ninth day of the study.
Table 1. Results of physical property test on field plots treated with the microbial composition.

<table>
<thead>
<tr>
<th>Test</th>
<th>Treated (T)</th>
<th>Control (C)</th>
<th>Replicates</th>
<th>Mean</th>
</tr>
</thead>
<tbody>
<tr>
<td>Modulus of Rupture</td>
<td>T</td>
<td>7</td>
<td>3.55*¹</td>
<td></td>
</tr>
<tr>
<td></td>
<td>C</td>
<td>7</td>
<td>4.52¹</td>
<td></td>
</tr>
<tr>
<td>Wet Aggregate Stability</td>
<td>T</td>
<td>9</td>
<td>46.0%*</td>
<td></td>
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<tr>
<td></td>
<td>C</td>
<td>9</td>
<td>35.8%</td>
<td></td>
</tr>
<tr>
<td>Field Penetration</td>
<td>T</td>
<td>20</td>
<td>94 lbs.*</td>
<td></td>
</tr>
<tr>
<td></td>
<td>C</td>
<td>20</td>
<td>103 lbs.</td>
<td></td>
</tr>
</tbody>
</table>

1. $10^{5}$ dynes/cm²

* Significant at 95% confidence level using 2-tailed t-test to compare means.
Table 2. Soybean emergence through field soil crust.

<table>
<thead>
<tr>
<th>Soybean Variety</th>
<th>Row No.</th>
<th>% Emergence Control</th>
<th>% Emergence Treated</th>
</tr>
</thead>
<tbody>
<tr>
<td>Stine 3560</td>
<td>1</td>
<td>81</td>
<td>83</td>
</tr>
<tr>
<td></td>
<td>2</td>
<td>67</td>
<td>75*</td>
</tr>
<tr>
<td></td>
<td>3</td>
<td>75</td>
<td>83</td>
</tr>
<tr>
<td>Williams 82</td>
<td>4</td>
<td>48</td>
<td>81</td>
</tr>
<tr>
<td></td>
<td>5</td>
<td>67</td>
<td>75*</td>
</tr>
<tr>
<td></td>
<td>6</td>
<td>56</td>
<td>77</td>
</tr>
</tbody>
</table>

1. One row equals 4 planter boxes.
2. Emergence at 21 days.
   * Significant at the 90% confidence level using the 2-tailed t-test for comparison of means.

Discussion and Conclusion: The modulus of rupture results were lower for the treated versus the control, indicating less potential for hard surface crusting and clod formation upon drying. The field penetrometer measurements supported the laboratory results for modulus of rupture, in pointing to a softer soil surface. The treated soils had greater water stable aggregate formations, which is generally considered favorable to crops because it indicates potential for higher water infiltration rates and improved aeration (Kemper, 1965) as well as reduced erosion. The emergence study indicated a crop response to soil treatment with the microbial composition. In this case the crop response was legume seed emergence, which is normally sensitive to the condition of the surface layer of the soil profile. The treated areas showed better emergence
performance over the non-treated area. The emergence results suggest a soil condition at the surface layer, produced by treatment effects, which is more conducive to emergence of soybean seed. The results in this test suggest grounds for the assumption that the microbial composition studied herein is of potential value in counteracting surface soil compaction, crusting and improving the tilth of silty clay loams for the Iowa, U.S.A., location of this test site. The test results further suggest the potential for “dry land” (non-irrigated) agricultural applications for the microbial composition.

Case Two: U.S.A.

Introduction: In this U.S.A. case study compaction data from 80 Southeast Iowa dry-land on-farm sites in 1986 and 1987, and 45 North Central Iowa dry-land on-farm sites in 1986 was collected to estimate the effect that the microbial soil inoculation (described under Methodology above) would have on soil structure. Yield data was collected from within the same sites where compaction data was collected. Corn yields were calculated in 1986 from 57 Southeast Iowa, and 9 North Central Iowa on-farm sites. Corn yields were calculated in 1987 from 39 Southeast Iowa on-farm sites. Soybean yields were calculated in 1987 from 37 Southeast Iowa on-farm sites.

Method: Two experimental regions were established for on-farm test sites in 1986 and one region in the 1987 crop year. Within a region there
are similar soil origins, cropping practices and climatic conditions. The more common textural soil type for the two regions is a silty clay loam, but the North Central Iowa region soils typically contain more organic matter than the soils in the Southeast Iowa region. The regions were defined as Southeast Iowa (north of Highway 34 and south of I-80 and east of Knoxville, Iowa) for 1986 and 1987 and North Central Iowa (north of Highway 3, west of Mason City and east of Spenser). The North Central Iowa region was utilized for only the 1986 crop year. Each region represented a single experimental area. Each test site was a cooperating farm-operator’s field. The operators applied the microbial composition, which consisted of microbes as defined in Methodology above, which are cells suspended and dispersed in a dry, clay based powder. This material was mixed with water and applied with conventional farm spray equipment at rates between 10 to 20 gallons per acre. The inoculation for each site was either before or after the planting of the principle crop. In each case for 1986 the treated fields were receiving their first application with the composition and received only one application for the 1986 measurement period defined herein. The same applies for the 1987 Southeast Iowa region except for corn, where 14 of 39 sites were also treated in 1986. For soybeans 16 of 39, 1987 sites, were also treated in 1986. For compaction measures 61 of the 80, 1987 sites, were also treated in 1986. The plots were paired with inoculated (treatment) and non–inoculated (control) areas side by side in each field.
The experimental measurement area was selected from within the treated and non-treated areas of each field. Each test or measurement area was 150 x 150 ft. with one half of the area the treated area and the other half within the non-treated area. Each measurement area was selected for uniformity of soil type, slope, drainage pattern and cultural history.

For compaction data collection a Soiltest bearing ring cone penetrometer was used. The cone had a cross sectional area of 2.54 cm² and had a 30 degree angle. Twenty subsample penetrometer probe data points were randomly selected, for each the treated and non-treated areas. Each of the 80 Southeast Iowa and 45 North Central Iowa sites were probed one time, 4 to 10 weeks after the composition treatment. The twenty subsample measurements were integrated to produce a mean for each the treated and non-treated areas for each set of paired plots. All measurements were adjusted to U.S. pounds/inch². Measurements were taken at the two and four inch depths. A one inch soil core to the four inch depth was taken at every tenth subsample data site for each set of paired plots in Southeast Iowa. Gravimetric moisture was analyzed on each of the soil samples. The two gravimetric subsample moisture analysis for each of the plots were integrated producing a percent moisture mean for each plot.

Regarding corn yield collection, for 1986 at 57 and 1987 at 39 Southeast Iowa and for 1986 at 8 North Central Iowa sites, six subsamples per treated and six per non-treated area for each paired plot.
were hand harvested, weighed, and shelled. Each subsample was equal to 1/1000 of an acre each. Grain moisture was determined and yield data was adjusted to a 15.5% grain moisture content. Yield data is in bushels per acre. The six subsamples for the treated area and six for the non-treated area for each paired plot were integrated to produce the mean for the treated area and the mean for the control area of each paired plot.

For soybean yield data collection, in 1986 at 44 and in 1987 at 37 Southeast Iowa and in 1986 at seven North Central Iowa sites, subsamples were collected per treated area and six for the non-treated area of each paired plot. Each subsample represented 1/1000 of an acre. Each subsample was hand harvested, air dried, weighed and adjusted to bushels per acre at 13% moisture content. The six subsamples per treated and control for each paired plot were integrated to produce a mean for each the treated and control area for the paired plot.

Each measurement area, a paired plot, containing a treated and non-treated control area, represented a replicate within a block. The statistical design was a randomized complete block, with each measurement area paired plot treated as a replication.

**Results:** The bearing ring cone penetrometer measurements for all test sites are presented in Table 1. The treated areas had significantly less penetrometer resistance to both the two, four and six inch depths. The soil water content difference between the treated and non-treated areas, calculated for Southeast Iowa only, was not significant.
The yield data collected at each site is presented in Table 2. A significant treatment effect was observed for 1986 and 1987 Southeast Iowa field corn yields. Southeast Iowa treated area yields averaged 8.8 bushels per acre greater than the non-treated areas for each 1986 and 1987. The 1986 North Central Iowa treated area, field corn yields, averaged an insignificant 5.9 bushels greater than the non-treated areas.

When 1987 Southeast Iowa field corn yield increases for test areas also treated in 1986 were compared to the same where only a 1987 treatment was given, the 2 years of treatment mean yield increase was 11.1 and the 1987 one year only yield increase mean was 7.3 bushels per acre. The extra yield increase for two years over one year of treatment was statistically insignificant.

Southeast Iowa treated soybean areas, averaged in 1986 a significant 4.4 bushels per acre greater than control areas. In 1987 the Southeast Iowa treated soybean areas averaged a significant 1.1 bushels per acre higher yield that the non-treated area. The 1986 North Central Iowa treated area soybean yields, averaged an insignificant 0.3 bushels greater yield than the non-treated areas.

The 1986 Southeast Iowa seed corn sites showed a 13.3 bushel larger yield on treated areas compared to non-treated areas, but the result was not statistically significant, primarily because of only two replicates for the “seed corn” category.
### Table 1. Bearing ring cone penetrometer measurements.

<table>
<thead>
<tr>
<th>Depth¹/year</th>
<th>Replicates/ Region²</th>
<th>Control Mean</th>
<th>% Moisture Mean</th>
<th>Treated Mean</th>
<th>% Moisture Treated</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>lbs./inch²</td>
<td>lbs./inch²</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>2/1986</td>
<td>80/SEIA</td>
<td>58.24</td>
<td>------</td>
<td>40.63*</td>
<td>------</td>
</tr>
<tr>
<td>2/1987</td>
<td>80/SEIA</td>
<td>45.79</td>
<td>------</td>
<td>33.57*</td>
<td>------</td>
</tr>
<tr>
<td>2/1986</td>
<td>45/NCIA</td>
<td>189.06</td>
<td>------</td>
<td>106.46*</td>
<td>------</td>
</tr>
<tr>
<td>4/1986</td>
<td>80/SEIA</td>
<td>125.78</td>
<td>16.0</td>
<td>95.29*</td>
<td>15.7***</td>
</tr>
<tr>
<td>4/1987</td>
<td>80/SEIA</td>
<td>117.79</td>
<td>------</td>
<td>94.23*</td>
<td>------</td>
</tr>
<tr>
<td>6/1987</td>
<td>80/SEIA</td>
<td>172.80</td>
<td>------</td>
<td>162.36**</td>
<td>------</td>
</tr>
<tr>
<td>8/1987</td>
<td>80/SEIA</td>
<td>225.10</td>
<td>16.8</td>
<td>214.88***</td>
<td>17.0***</td>
</tr>
</tbody>
</table>

1. Inches of depth measured with penetrometer.
2. Regions are SEIA = Southeast Iowa and NCIA = North Central Iowa.
   * Significant at the 0.01 probability level.
   ** Significant at the 0.05 probability level.
   *** Insignificant
Table 2. Crop yield measurements on all yield collection sites by crop, region and year.

<table>
<thead>
<tr>
<th>Year/Crop</th>
<th>Replicates/Region¹</th>
<th>Control Bushels/Acre</th>
<th>Treated Bushels/Acre</th>
</tr>
</thead>
<tbody>
<tr>
<td>1986/Field Corn</td>
<td>57/SEIA</td>
<td>167.7</td>
<td>176.5*</td>
</tr>
<tr>
<td>1987/Field Corn</td>
<td>39/SEIA</td>
<td>158.0</td>
<td>166.8*</td>
</tr>
<tr>
<td>1986/Field Corn</td>
<td>8/SEIA</td>
<td>166.6</td>
<td>172.5**</td>
</tr>
<tr>
<td>1986/Soybeans</td>
<td>63/SEIA</td>
<td>45.5</td>
<td>49.9*</td>
</tr>
<tr>
<td>1987/Soybeans</td>
<td>37/SEIA</td>
<td>49.6</td>
<td>50.7*</td>
</tr>
<tr>
<td>1986/Soybeans</td>
<td>7/NCIA</td>
<td>37.3</td>
<td>37.6**</td>
</tr>
<tr>
<td>1986/Seed Corn</td>
<td>2/SEIA</td>
<td>48.2</td>
<td>61.5**</td>
</tr>
</tbody>
</table>

¹ Regions are: SEIA = Southeast Iowa and NCIA = North Central Iowa.

* Significant at the 0.01 probability level.

** Insignificant.

**Discussion and Conclusion:** Certain soil organisms produce polysaccharides that have a mucilaginous nature and may cement soil mineral particles together. Rennie et al. (1954) found that the addition of only 0.02g of an extracted and purified soil polysaccharide material to 100g of soil increased the water-stable aggregates > 0.1mm in diameter from 44g to 60g. Chesters et al. (1957) on analysis of a large number of soils, indicated that the per unit mass, the polysaccharide fraction was more effective in stabilizing the mineral particles into structural units than was the non-polysaccharide portion of the soil organic matter.
In experimental work, it is frequently desirable to evaluate soil structure issues because the most suitable management practices may depend on the extent to which structure affects the growth of plants. With the advent of synthetic, organic soil-aggregating agents, having only small direct effects on the microbiological population and nutrient status of soils, structural effects on crop performance could be measured. It has been observed that in some cases the improvement of structure through field application of a synthetic organic soil-aggregating agent did not improve crop yields (Clement, 1961) and in other examples a definite crop yield increase could be attributed to structural improvements associated with the addition of the aggregating agent (Boekel, 1963). It is generally agreed that improved soil structure is beneficial to soil as a medium for plant growth but observation dictates that not all crops, especially under various soil types, will respond to structural improvement with crop yield increases.

In this study, within one crop season, with one treatment, with a composition of microalgae and cyanobacteria, known to produce polysaccharides, the measurement of force required to displace or shear soil with a bearing ring cone penetrometer was significantly reduced at the two, four and six inch depths, (Table 1). The measurement of a greater difference from the zero to the two inch depth versus the two to the four, six or eight inch depths, is probably due to the majority of the microbial produced, organic, soil aggregating agent being tied up with the
soil mineralogy in the upper zone of the measurement depth. Each test site used a different type and design of farm spray equipment to spray soils with the product. The total number of sites in the experiment was high which should have minimized any bias in the data due to inconsistencies in the farm equipment calibrations. There were only two moisture subsamples for each the treated and non-treated area in each paired plot. However, the total number of moisture evaluations was high and the moisture bias in the penetrometer calculations was statistically insignificant, therefore it is reasonable to conclude that the differences in penetrometer measurements were not produced by differences in moisture content between treated and control areas.

Comparative yield increases associated with treatment indicates a possibility for yield improvements in the geographical area of this experiment. Southeast Iowa showed a greater yield response from the treatment compared to North Central Iowa. Soils in North Central Iowa tend to be higher in organic matter than those of Southeast Iowa, suggesting a greater need in Southeast Iowa for improvement in soil structural conditions, when compared to North Central Iowa.

The data in this test appear to produce a correlation between the treatment of a soil surface with a microalgal and cyanobacteria microbial composition, known biological polysaccharide producers, and decreases in surface soil compaction and improvement in crop yields. This observation is in keeping with those of Barber (1959) where in Indiana a higher
aggregation index, indicating less soil compaction, was observed to be closely associated with larger corn crop yields. It should be noted, however, that with biological sources for organic soil aggregating agents there may be other contributing factors benefiting the growing plant in addition to structural improvements (Clement, 1961). Therefore, it may be impossible to determine empirical relationships, direct or indirect, between soil structural improvements from biologically produced organic soil-aggregating agents and crop responses. It is however suggested by this work that a crop response may be associated with the treatment of soils with a composition of active polysaccharide producing microbial agents, especially for those soils identified to be in greater need of structural improvement.

**Case Three: U.S.A.**

**Introduction:** The Upper Mississippi Delta of the U.S.A. was selected as the region for the on-farm test sites. The sites within the region had similar soil origins, cropping practices and climactic conditions. The region is defined as Upper Delta, specifically encompassing in this case western Tennessee, southeast Missouri, eastern Arkansas, and northwest Mississippi. Each test site was a cooperating farm-operator’s dry-land field. Soils in the Upper Mississippi Delta region are considered suitable for crop production, but are generally of a lower quality when compared to Iowa soils in the U.S.A. with respect to their capacity to carry a crop.
Method: The treatment was the microbial composition described in Methodology above. This dry, powdered suspension of microalgae and cyanobacteria was mixed with water and applied with conventional farm spray equipment at rates between 5 to 20 U.S. gallons per acre. The treatment applications were done either before or after the planting of the principle crop. In each case the treated fields were receiving their first application with the microbial composition and received only one application for the 1987 measurement period. A treated and non-treated paired plot measurement area was located side by side within each field. This experimental measurement area was 150 x 150 ft. with one half of this area forming the treated plot and the other half forming the control plot. Each measurement area was selected for uniformity of soil type, slope, drainage pattern and cultural history.

For compaction data collection a Soiltest bearing ring cone penetrometer was used, the same as described in Case One and Two above. The penetrometer had a cross sectional area of 2.54cm² and a 30 degree angle. Twenty subsample penetrometer data sites were randomly selected, for each the treated and control in the paired plot configuration. Each of the 40 sites were probed one time, 4 to 10 weeks after the application of treatment. The twenty subsample measurements were integrated to produce a mean for each the treated and control paired plots and all penetrometer readings were adjusted to U.S. pounds/inch². Measurements were taken at the two and four inch depths. A one-inch
wide soil core to the four-inch depth was taken at every fourth subsample
data site within each plot. These soil cores were analyzed for gravimetric
moisture. The gravimetric subsample moisture readings for each plot
were integrated producing a percent moisture mean for the plot.

Regarding cotton yield collection within the region, during 1987, at 30
sites, six subsamples per plot, equal to 1/1000 of an acre each, were
estimated for yield, using a hand boll count method. All harvestable bolls
within each 1/1000 of an acre subsample were counted. Each boll count
was converted to an estimated yield per acre by the following formula:

\[
\frac{\text{# bolls}}{1/1000 \text{ acre}} \times 200 \times 1,000 = \text{lbs. lint/acre}
\]

All six subsample yield estimates per plot were integrated to produce an
estimated cotton yield mean for each plot.

Each set of paired plots represented a replicate. The statistical design
was a randomized complete block.

**Results:** The bearing ring cone penetrometer measurements for all sites
are presented in Table 1. The treated areas had significantly less
penetrometer resistance at both the two and four inch depths. The soil
water content difference between the treated and control paired plots,
across the region was not significant.

The yield data collected is presented in Table 2. A significant
treatment effect was observed for the estimated cotton yields. The treated
plots estimated yield averaged 115.1 pounds greater lint cotton than the control plots.

Table 1. Bearing ring cone penetrometer measurements at the two and four inch depths.

<table>
<thead>
<tr>
<th>Depth Inches</th>
<th>Replicates</th>
<th>Lbs./inch² Control</th>
<th>% Moisture Control</th>
<th>Lbs./inch² Treated</th>
<th>% Moisture Treated</th>
</tr>
</thead>
<tbody>
<tr>
<td>2</td>
<td>40</td>
<td>71.67</td>
<td>------</td>
<td>47.96*</td>
<td>------</td>
</tr>
<tr>
<td>4</td>
<td>38</td>
<td>193.30</td>
<td>11.90</td>
<td>156.98*</td>
<td>12.08**</td>
</tr>
</tbody>
</table>

* Significant at the 1% level.
** Insignificant at the 5% level.

Table 2. Cotton yield estimates from the region.

<table>
<thead>
<tr>
<th>Replicates</th>
<th>Lint/Acre Control</th>
<th>Lint/Acre Treated</th>
</tr>
</thead>
<tbody>
<tr>
<td>30</td>
<td>1046.6</td>
<td>1161.7*</td>
</tr>
</tbody>
</table>

* Significant at the 1% level.

Discussion and Conclusion: The results of this field trial work indicate a crop response similar to the same observed in Iowa, presented in Case Two above. Within one crop season, one application of the microbial composition decreased surface horizon soil compaction as measured by penetrometer readings. It is highly likely that the measurement of a greater comparative reduction at the two inch depth, versus the four-inch depth, is due to the majority of the microbial produced soil-aggregating
polysaccharide being tied up with the soil mineralogy in the upper zone of
the measurement depth.

Estimated comparative cotton yield increases, associated with
treatment, indicates a possibility for cotton yield improvements in the
local region of this experiment, The data suggest a correlation between
compaction reductions associated with treatment and cotton crop
responses indicated by an average yield increase across the region. As
stated in Case Two above, this observation is in keeping with those of
Barber (1959) where in Indiana greater soil aggregation was observed to
be closely associated with larger corn crop yields.

Case Four: Japan

Introduction: This study was conducted in Japan during the period of
March 1988 to May 1989. Japan is a country where the recreational sport
of golf is very popular. With this in mind, the crop choice for this test was
turf grass. The test was designed to be a small scale simple assay to
evaluate the effectiveness of the microbial composition as an application to
turf sod, with respect to improvement of physical properties of the soil
under the turf commonly related to particulate aggregation and
determination of a turf grass response associated with treatment.

Methods: The test was conducted at Higashi-ku, Fukuoka City, Japan.
The field was owned by the South Japan Green Institute. The size of the
test area was 4 m². Eight plots were employed in the experimental design.
Plots were randomly placed in the block. The variety of the turfgrass was Kahrai Shiba (*Zoysia matrella merr.*). The type of soil was a weathered granite sand. There were two control plots, two plots treated two times with the microbial composition (as described above in the Methodology section), two plots treated three times with the composition and two plots treated three times where herbicide was also applied to see if herbicide application might have an adverse effect on the results. The 1988 treatment dates were: March 18, May 30 and September 13. The application dates for the herbicide were: April 19 and October 15. All plots received conventional fertilization.

The resistance to penetration (soil hardness) was measured by using a penetration meter from Yamanaka (Japan manufacturer). This measure was determined for each plot nine different times during the experimental period. At each measurement interval, there were five subsample data points per plot and they were integrated to produce a mean for each plot. A three phase distribution, a measure of soil porosity (air/water to soil ratio), was determined five different times during the term of the experiment to measure the relationship between gas and liquid in the soil profile. The standard volumetric method (100cc) was utilized. Water permeability rate was measured at two intervals using a PVC pipe (100 mm diameter by 250 mm length) which was driven into the soil up to 50 mm deep. Then the upper vacant area of the pipe was filled with water. The amount of water infiltrated after one hour was measured.
There were three such subsample evaluations per plot and the three measures were integrated to determine a mean for the plot. The harvested fresh weight of the top growth of the grass plots was determined at three different intervals during the measurement period and reported as “per square meter”. The plant root weights were evaluated at two different times during the period of the experiment. The root weights were determined by taking two subsamples per plot at the 200 mm depth by hole cutter. The roots were gently washed by water to remove soil. The root material was removed from the plant. The roots were air dried and weighed. Subsamples were integrated to produce a mean result and were adjusted to report as per square meter.

The design incorporated only two replicates per variable, making statistical analysis difficult, therefore there was no such analysis applied against the data.

Results: The results for penetration (soil hardness) are presented in Table One. The treated plots displayed less resistance in the penetration measure through out the measurement period. The results for the three-phase distribution are presented in Table Two. The results show a slight increase in soil porosity, especially at the end of the 1988 measurement period. The results for water infiltration rate are presented in Table Three. The plots treated three times with the microbial composition showed a marked increase in water infiltration rates. The plots treated only two times did not show an increase in infiltration rate for water when
compared to the control plots. The results for measure of top growth fresh grass growth are presented in Table Four. The data for this measure displays a strong trend in favor of treatment with respect to increased top growth of the turf grass. The data for measure of dry root weight is presented in Table Five. Again, the dry root weight measure shows a strong trend in favor of treatment for all three of the treated plot variables.

Table 1. Soil penetration resistance (mg/cm³).

<table>
<thead>
<tr>
<th>Dates &gt;</th>
<th>3/18/88</th>
<th>4/11</th>
<th>5/7</th>
<th>5/30</th>
<th>6/23</th>
<th>7/13</th>
<th>8/5</th>
<th>9/10</th>
<th>10/15</th>
<th>11/16</th>
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<td></td>
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</tr>
<tr>
<td>A¹</td>
<td>15.2</td>
<td>15.4</td>
<td>14.9</td>
<td>17.0</td>
<td>15.6</td>
<td>15.0</td>
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<td>14.7</td>
<td>16.1</td>
<td>15.2</td>
<td>15.7</td>
<td>15.7</td>
<td>16.1</td>
<td>15.8</td>
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<tr>
<td>B²</td>
<td>13.7</td>
<td>13.5</td>
<td>13.3</td>
<td>16.5</td>
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<td>13.0</td>
<td>12.9</td>
<td>14.8</td>
<td>15.3</td>
<td>15.1</td>
<td>15.5</td>
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<td>C³</td>
<td>15.5</td>
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<td>15.1</td>
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<td>14.6</td>
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<td>14.9</td>
<td>15.9</td>
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<td>15.8</td>
<td>15.0</td>
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</tr>
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<td>15.3</td>
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<td>18.5</td>
<td>18.9</td>
<td>17.6</td>
<td>17.5</td>
<td>17.0</td>
</tr>
</tbody>
</table>

1. A is three treatments.
2. B is three treatments plus herbicide treatments.
3. C is two treatments.

Table 2. Three phase distribution (percentage porosity).

<table>
<thead>
<tr>
<th>1988 Dates &gt;</th>
<th>5/10</th>
<th>6/28</th>
<th>9/20</th>
<th>11/16</th>
</tr>
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<tbody>
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<td>Categories</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>A¹</td>
<td>51.7%</td>
<td>24.6%</td>
<td>23.7%</td>
<td>48.3%</td>
</tr>
<tr>
<td>B²</td>
<td>50.9</td>
<td>26.5</td>
<td>22.6</td>
<td>49.1</td>
</tr>
<tr>
<td>C³</td>
<td>50.5</td>
<td>22.9</td>
<td>26.6</td>
<td>49.5</td>
</tr>
<tr>
<td>Control</td>
<td>52.7</td>
<td>21.2</td>
<td>26.1</td>
<td>47.3</td>
</tr>
</tbody>
</table>

1. A is three treatments.
2. B is three treatments plus herbicide treatments.
3. C is two treatments.
Table 3. Water infiltration rate (mm/hr).

<table>
<thead>
<tr>
<th>Categories</th>
<th>11/18/88</th>
<th>4/28/89</th>
</tr>
</thead>
<tbody>
<tr>
<td>A¹</td>
<td>26.0</td>
<td>32.0</td>
</tr>
<tr>
<td>B²</td>
<td>31.0</td>
<td>28.0</td>
</tr>
<tr>
<td>C³</td>
<td>12.5</td>
<td>20.5</td>
</tr>
<tr>
<td>Control</td>
<td>17.5</td>
<td>21.0</td>
</tr>
</tbody>
</table>

1. A is three treatments.
2. B is three treatments plus herbicide treatments.
3. C is two treatments.

Table 4. Fresh top growth weights (g/m²).

<table>
<thead>
<tr>
<th>Categories</th>
<th>6/25/88</th>
<th>9/20/88</th>
<th>5/1/89</th>
</tr>
</thead>
<tbody>
<tr>
<td>A¹</td>
<td>278.1</td>
<td>82.4</td>
<td>59.2</td>
</tr>
<tr>
<td>B²</td>
<td>282.3</td>
<td>94.9</td>
<td>73.1</td>
</tr>
<tr>
<td>C³</td>
<td>223.9</td>
<td>86.8</td>
<td>73.2</td>
</tr>
<tr>
<td>Control</td>
<td>187.1</td>
<td>69.4</td>
<td>69.4</td>
</tr>
</tbody>
</table>

1. A is three treatments.
2. B is three treatments plus herbicide treatments.
3. C is two treatments.
Table 5. Root dry weights (g/m²).

<table>
<thead>
<tr>
<th>Categories</th>
<th>6/27/88</th>
<th>5/1/89</th>
</tr>
</thead>
<tbody>
<tr>
<td>A</td>
<td>22.6</td>
<td>33.5</td>
</tr>
<tr>
<td>B</td>
<td>23.1</td>
<td>41.6</td>
</tr>
<tr>
<td>C</td>
<td>23.1</td>
<td>27.7</td>
</tr>
<tr>
<td>Control</td>
<td>18.5</td>
<td>23.7</td>
</tr>
</tbody>
</table>

1. A is three treatments.
2. B is three treatments plus herbicides.
3. C is two treatments.

Discussion and Conclusion: The results of this assay on Japanese sandy soil conditions suggest agronomic advantages associated with treatment. The resistance to penetration data indicate that the treatment reduced soil compaction under turf grass sod conditions. The effect of reduced compaction, as a data trend, was most noticeable after two treatments had been administered. There were no extra advantages observed when three treatments were compared to two. The effect of reduced compaction continued to be present in the data in the spring season for the year following treatment, suggesting a carry over from the year of treatment to the year after treatment. Soil hardness tended to increase on the control with respect to time by comparison to the treatments where the measure remained relatively constant with respect to time.

In the three-phase distribution test the ratio of gas and liquid, against solids, a measure of the soil porosity ratio, increased from 2.1 to 4.7%.

This does not suggest a large increase in porosity. On the other hand, the
specific ratio for the liquid phase did show an increase, which suggest an increase in water infiltration or holding capacity.

In the case of water infiltration measurement there was a marked improvement in both the variables which employed three treatments with the composition. This type of result is commonly associated with the measure of less compaction, in that compaction of soil will tend to decrease water infiltration rates, while decreasing surface soil compaction normally improves water infiltration.

The measure of top growth production for this specific type of Zoysia turf grass, common on the Japanese golf course, showed a marked increase in biomass generation. This effect was noticed in the data starting about two months after the first application of the microbial composition. At the investigation on June 25, all the treated sites had a better yield, compared to the control, by 20 – 51%. This observation was present in the data, with respect to time in the experimental period, prior to the observation of improvement in soil conditions. This suggest a direct plant stimulating effect associated with treatment, perhaps in addition to the indirect benefits to the plant corresponding to soil structural change. This observation continued into the fall month of September. There was no indication in the data that three applications were any better than two applications with respect to increasing plant top growth production. At the beginning of May, 1989, this Spring measure indicated no advantage in favor of treatment, however root growth was better on this same date,
suggesting a basis for speculation that at a later date in the spring season the top growth measure associated with treatment may also be greater than that associated with the control.

The root growth was measured two times in this experiment. The first measure was done in June, 1988. At this time the increase in root growth was moderate, but consistent across all three treatment variables, against the control. At this date there was no difference between two or three treatments with the microbial composition. At the second measurement date, May 1989, there was a large increase in root mass associated with treatment, when compared with the control. For this date there was a noticeable advantage associated with three treatments compared to only two. It seems that the plant response to any carry over effects from 1988 to 1989 was first and foremost, at least at the date of May 1, 1989, to be found in the root biomass. Soil compaction decreased under treatment in this experiment, especially with respect to time. There may be a correlation in the improvement of root growth, especially noticeable at the end of the measurement period, to the decrease in soil compaction. Root development is generally decreased in a soil profile which is compacted, but may increase where soil compaction in the root zone is decreased.

In this study there was no noticeable negative effect from the application of herbicides on treated plots. The herbicide treatments were scheduled one month after application of the microbial composition. This
result suggest that the one month interval is an adequate safe guard with respect to any potential need to avoid herbicide injury to the microbial composition.

The improvements in soil physical properties in this Japanese assay seem to be correlated with the turf response. This suggest, as other investigators have observed, that crop response is influenced by the soil structural condition. Further it suggest that a treatment with the microbial composition utilized in this experiment is of agronomic value to Zoysia turf grass grown on weathered sandy soils in Japan. It seems the golf course industry of Japan could take advantage of this microbial composition to address soil structural problems common to the golf course environment.

Case Five: Australia

Introduction: The purpose of this Australian trial is to evaluate the yield effect on irrigated cotton associated with soil treatment with the microbial composition described above under Methodology. The production of cotton is a major agricultural cropping enterprise in Australia. This trial was conducted in the Macquarie Valley, near Trangie, New South Wales, Australia. The soils in this valley, as is the case for Australia in general, are considered marginal with respect to their capacity to carry crops.

Methods: The trial was established on the farm of a cooperating cotton grower. The cropping year was 1992/93. No pre-plant herbicides were
used for the cotton crop. Herbicides were used over the plant line at planting. There were seven control and seven treated plots, organized as paired plots. Plot size was 10 rows wide by 60 m long. The actual measure of plot width was 7.5 m. The control and treated plots were aligned alternately across the field. The plots (measurement areas) were kept 20 m from the end of the field row or 20 m from the irrigation head ditch. The total size of the trial was 0.9 hectare.

The treatment with the microbial composition was done three weeks prior to planting. The dosage level for the treatment was the same as that described in Methodology above, 70 grams per hectare. The composition was weighed and then mixed in a slurry and added to fresh water in a saddle tank mounted on the front of the cooperating farmer’s tractor. This mixture was agitated using the return system also mounted on the tractor, driven by a hydraulically powered pump. More water was added and the mixture was agitated further.

Application of this mixture to the soil was done through spray lines and nozzles mounted to the farmer’s cultivator, normally used for band spraying when cultivating. When spraying the treated area, the cultivator was not engaged into the soil but set at a height that allowed 100% cover of the ground with some overlapping of the spray mist. With this procedure it was presumed that all the ground surface was treated with equal amounts of the microbial composition treatment.
The treatment was applied to the bare moist soil surface. No incorporation was used when applying the treatment. The treatment was applied in 150 liters/ha of fresh, unchlorinated water. The pressure produced by the tractor mounted hydraulic pump was 160 kpa. Air temperature at the time of application was 24.5 degrees C and air humidity was 39%. Because the trial site was within 20 m of the head ditch, the tractor and application equipment mounted to the cultivator were reversed into the field, well past the outside of the border of each plot to be treated, and then the spraying was done on the way out of the field. Reversing well past the outside of each plot allowed sufficient distance for the operating speed of the tractor to be reached before spraying commenced. Fifteen millimeters of rain fell the night following application of the composition. There was no cultivation disturbance of the soil in the plots between the application date and the planting date. The cotton seed was planted on October 14, 1992. The variety of cotton planted was Siokra 1-4.

For yield estimation purposes, hand harvesting of 4 x 1m subsamples was conducted in the same row in each plot. The row chosen was number two. This avoided any problems of compaction that may have resulted during the application of the treatment or subsequent planting and cultivating operations. Hand harvesting took place on May 12, 1993. The number of plants in each meter of row was recorded and so too was the
number of open bolls harvested. The 4 individual subsamples per plot were grouped together for each plot and weighed on an electronic scale.

Results: The results of the plant stand count are presented in Table One. The treatment produced a mean for all replicates greater than the control, but the result was not significant. Of the seven paired plots, only three showed an increase in plant stand associated with treatment. The harvestable bolls count is presented in Table Two. The treatment produced a mean for the seven treated plots which was greater than the mean for the seven control plots. The result was significant. The increased boll count associated with treatment was measured in six of the seven paired plots. The result for the seed cotton weight is presented in Table Three. The treatment produced a greater mean seed cotton weight for the seven plots compared to the seven control plots. The result was significant. The increase in seed cotton weight associated with treatment was observed in six of the seven paired plots.
Table 1. Established plant stand counts¹.

<table>
<thead>
<tr>
<th>Paired Plot No.</th>
<th>Control²</th>
<th>Treated²</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>10.75</td>
<td>12.0</td>
</tr>
<tr>
<td>2</td>
<td>10.0</td>
<td>9.5</td>
</tr>
<tr>
<td>3</td>
<td>9.5</td>
<td>13.25</td>
</tr>
<tr>
<td>4</td>
<td>10.75</td>
<td>9.8</td>
</tr>
<tr>
<td>5</td>
<td>12.25</td>
<td>14.25</td>
</tr>
<tr>
<td>6</td>
<td>11.5</td>
<td>10.5</td>
</tr>
<tr>
<td>7</td>
<td>9.5</td>
<td>9.5</td>
</tr>
<tr>
<td>Mean</td>
<td>10.6</td>
<td>11.25*</td>
</tr>
</tbody>
</table>

¹. Per meter.
². Four subsamples taken per plot and integrated to produce a mean for the plot.
* Not significantly different.
Table 2. Open harvestable bolls\(^1\).

<table>
<thead>
<tr>
<th>Paired Plot No.</th>
<th>Control(^2)</th>
<th>Treated(^2)</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>80.3</td>
<td>97.5</td>
</tr>
<tr>
<td>2</td>
<td>88.5</td>
<td>82.75</td>
</tr>
<tr>
<td>3</td>
<td>79.3</td>
<td>94.5</td>
</tr>
<tr>
<td>4</td>
<td>79.8</td>
<td>111.8</td>
</tr>
<tr>
<td>5</td>
<td>84.0</td>
<td>95.75</td>
</tr>
<tr>
<td>6</td>
<td>77.8</td>
<td>91.0</td>
</tr>
<tr>
<td>7</td>
<td>79.3</td>
<td>93.0</td>
</tr>
<tr>
<td>Mean</td>
<td>81.3</td>
<td>95.2(^*)</td>
</tr>
</tbody>
</table>

1. Per meter.
2. There were four subsamples taken per plot and integrated to produce a mean for each plot.

\(^*\) Significant using a single factor analysis of variance and by the calculation of least significant differences at the 5\% probability level.
Table 3. Seed cotton weights¹.

<table>
<thead>
<tr>
<th>Paired Plot No.</th>
<th>Control²</th>
<th>Treated²</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>1411.2</td>
<td>1644.0</td>
</tr>
<tr>
<td>2</td>
<td>1524.4</td>
<td>1410.0</td>
</tr>
<tr>
<td>3</td>
<td>1298.0</td>
<td>1578.2</td>
</tr>
<tr>
<td>4</td>
<td>1334.8</td>
<td>1952.8</td>
</tr>
<tr>
<td>5</td>
<td>1424.0</td>
<td>1548.8</td>
</tr>
<tr>
<td>6</td>
<td>1327.6</td>
<td>1449.6</td>
</tr>
<tr>
<td>7</td>
<td>1328.3</td>
<td>1480.6</td>
</tr>
<tr>
<td>Mean</td>
<td>1378.3</td>
<td>1580.6*</td>
</tr>
<tr>
<td>Total³</td>
<td>9,648.3</td>
<td>11,064.4</td>
</tr>
<tr>
<td>Bales³</td>
<td>7.29/ha</td>
<td>8.35/ha</td>
</tr>
</tbody>
</table>

1. Grams per 4 meters.
2. There were four one-meter subsamples taken per plot and totaled to produce a four-meter total for each plot.
3. Total for control and treated, utilized in calculating the data in terms of bales per hectare. See yield calculation below.

* Significant using a single factor analysis of variance and by the calculation of least significant differences at the 5% probability level.

Yield Calculations:

Control: \((9648.3 \text{ g/7 plots}) \times 36\% \text{ out turn}\)

\[
= 496.20 \text{ g/4 meter row} \\
= \frac{(496.20 \text{ g/4 meter row})}{0.75 \text{ rows/m}} \times \frac{10,000 \text{ m}^2/\text{ha}}{1,653,994 \text{ g/ha lint} / 1000 \text{ g/kg} / 227 \text{ kg/bale}} \\
= 7.29 \text{ bales/ha or 2.91 bales/acre}
\]

Treated: \((11,064.4 \text{ g/7 plots}) \times 36\% \text{ out turn}\)

\[
= 569.03 \text{ g/4 meter row} \\
= \frac{(569.03 \text{ g/4 meter row})}{0.75 \text{ rows/m}} \times \frac{10,000 \text{ m}^2/\text{ha}}{1,896,767 \text{ g/ha lint} / 1000 \text{ g/kg} / 227 \text{ kg/bale}} \\
= 8.35 \text{ bales/ha or 3.34 bales/acre}
\]
**Discussion and Conclusion:** Improving trends existed in all parameters measured. The application of the microbial composition improved plant stand, the number of harvestable bolls and final yield estimations. The difference in plant stand count was the only parameter that was not significantly improved. The final yield was increased by 202.3 grams of seed cotton per 4 meters or 1.06 bales per hectare. This suggest practical agronomic applications for the microbial composition in New South Wales, Australia, with respect to the production of cotton. With few exceptions, Australia is basically void of high organic matter soils. Soils in the New South Wales area are perhaps better than most in the country, but in general would be classed by world standards as marginal with respect to their capacity to carry a crop. As in the U.S.A. cases, described above, the microbial composition may have its more practical application on soils which are more marginal with respect to their capacity to carry cropping operations.

**VII. FINAL CONCLUSION:**

In this research work the focus has been to test and demonstrate the effectiveness of a cyanobacteria and microalgae microbial composition as soil inoculants, the purpose being the modification of soil physical properties and the generation of a crop response. This work was organized as an international effort, located on two continents and one sub-continent. In selecting diverse geographical locations it is possible to
suggest a certain universal potential for the agronomic application of the microbial composition.

Test results may be summarized as follows:

1. In the U.S.A., test indicated a significant change in soil physical properties through the measure of improved soil aggregate stability, decreased strength as measured by modulus of rupture and decreased soil compaction. Related to these observations was the improvement in soybean seedling emergence under treated soil conditions. These indicators are of agronomic value, because they suggest that the microbial composition was effective in improving the soil structural condition. Improved soil structural condition is of value in preserving the top soil of agricultural fields (poor top soil structural condition is known to be associated with the potential for soil erosion). Improved aggregate stability may help reduce the incidence of soil wind and water erosion events in an open field environment. Improvements in soil aggregate structural condition and the corresponding improvements in seedling emergence may increase plant population for a field crop, which may be valuable in increasing crop yields. Better management of the soil condition is of agronomic importance in sustaining productivity levels for the current time and for generations to come.

2. The Japanese test also demonstrated a strong corollary between soil physical property modification and plant response. In this assay the
data indicated soil modifications, including less compaction and improved water infiltration rates. Associated with these soil physical property modifications were measures of increased turf grass top growth and root biomass. This association, soil modification and plant biomass response, provide an important insight as to the dynamics of treatment with respect to the microbe-soil-plant interface. With the removal of soil physical impediment to root development (decreased compaction), plus improvement in water intake, a plant may respond with greater root development and a corresponding top growth enhancement.

3. Also, in the U.S.A., there were measures of improved corn and soybean yields. In this study the overall typical corn yield increase in the state of Iowa was 7.8 bushels per acre. Assuming a common corn value of $2.25 per bushel, this represents an extra $18 per acre. Considering the average yield for controls in the Iowa study, at 166 bushels per acre, and a value of $2.25 per bushel, the extra potential gross income, associated with treatment, represents an increase of 4.8%. Assuming a cost of $5 per acre for treatment, the extra potential gross income per acre represents a 3.6:1 return on investment ratio. Considering that Iowa corn production is today a highly competitive, low margin, commodity cropping operation, increases in potential income of 4.8% and return ratios of 3.6:1 is suggested to be a reasonable return on investment. The soybean plant
response in Iowa showed an overall average increase in yield of 1.9 bushels per acre. Assuming the on-farm value of soybeans is worth $6 per bushel, this extra yield has an estimated value of $11. Assuming a cost for treatment at $5 per acre, the extra income per acre represents an estimated 2.2:1 return on investment. The control overall average yield in the Iowa test was 44.1 bushels per acre or an estimated value of $265. The extra potential $11 per acre gross income represents a 4.1% increase in gross income. For commodity crop production, this is suggested to be a practical rate of return. It is suggested by the data that the utilization of the microbial composition, as a method to improve a soil structural condition, which indicates potential for less soil loss and damage, while at the same time generating a profit from treatment, is a practical approach to sustaining the productive capacity of soils in Iowa.

4. Another study in the Upper Mississippi Delta of the U.S.A. showed reduced compaction and an increase in cotton yield. In this case the cotton yields were 1046.6 lbs. for the control and 1161.7 lbs. for the control per acre. The increase in yield was 115.1 lbs per acre. Assuming an on-farm market value of $0.55 per lb. for lint cotton, the increase in yield had an estimated value of $63 per acre. Against an anticipated cost of $5 per acre for treatment, this is a return ratio of 12.6:1. The control cotton yield in this study had an estimated value of $576 per acre. The extra potential $63 represents a 10.9% increase
in gross income per acre. This estimation indicates a practical economic value associated with treatment of cotton fields in the Upper Mississippi Delta of the U.S.A., while at the same time the soil structural condition showed improvement.

5. In Australia, similar to results in the U.S.A. with respect to crop yield response to treatment, data indicate a practical application in the business of producing cotton. Cotton yields were increased with treatment from 7.29 bales to 8.35 bales per hectare. A bale is 227 kg. A typical on-farm value per kg of cotton is US$1.21. The extra cotton associated with treatment was 240.62 kg per hectare. This equals an estimated value of $291 per hectare. The anticipated cost of treatment per hectare in Australia is $20 (including material plus importation cost factors). The extra cotton yield in this study represents an estimated 14.55:1 return on investment. The gross estimated value of the cotton for the control per hectare was $2004. The extra $291 per hectare, associated with treatment, suggest a potential increase in gross income of 14.5%. This data indicates a practical economic value, associated with the treatment of cotton fields in Australia with the microbial composition used in this research.

The data presented herein suggest that the microbial composition may have applications of agronomic value on a diverse geographical scale. The results of this study indicate a potential for agronomic applications for the treatment in systems of dry-land and irrigated crop
production. The research design incorporated multiple crops and turf grass applications, suggesting that the microbial composition may have practical value for a diverse array of crops and non-crop plants, including both grasses and legumes. The data suggested that the treatment was of greater value in production zones where soils were more moderate or poor with respect to their structural and chemical integrity and associated capacity to carry a crop.

Additional research is indicated by the results in this study. Suggested areas of future research are:

1. Continued testing of the microbial composition on different soil types, in different areas of the globe and more test, within countries of choice, favoring those soils which are more moderate or poor with respect to their capacity to carry a crop.

2. Further test of the treatment on other types of crops and more test on the crops utilized in this study.

3. Work should be conducted on the soil chemistry associated with treatment, especially focusing on the polysaccharide component of the soil and the interface between treatment contributed polysaccharide and the soil structural matrix.

4. Additional work should be conducted to further documentation of the soil physical property changes that occur in the soil matrix associated with treatment.
5. The effects of the treatment should be studied in the context of additional practical agronomic applications, such as efforts to control wind and water soil erosion. It is suggested that such measures should be reduced to practical economic determinations.

The research results in this study indicate practical agronomic possibilities for a management contribution to the soil resources of the world, utilizing a microbiological soil inoculant strategy based on cyanobacteria and microalgae. This work suggest the potential to employ sustainable, ecologically safe techniques to address soil related problems in global agriculture, while at the same time producing immediate economic reward for the grower. It seems practical and efficient for the global agricultural community to consider methods, such as the method demonstrated in this study, which might address important soil related natural resource management concerns, while at the same time improve the profitability of farming operations. It is suggested by this work that the future of agriculture is best preserved by favoring the more sustainable approach to problem solving, as demonstrated herein.