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**ABSTRACT**

There is provided an aqueous seaweed extract in the form of a solution concentrate composition comprising:

- (a) at least 0.5 % by weight soluble alginate based on the weight of concentrate composition;
- (b) at least 0.1% calcium by weight based on the weight of concentrate composition;
- (c) a calcium-chelating agent; and
- (d) a borate compound.

## **Aqueous Seaweed Extract in the Form of a Solution Concentrate**

### **Field**

- 5 [0001] This invention relates to a liquid seaweed extract in concentrated form comprising alginate and calcium ions, to methods of preparing such concentrates and to their use in agriculture particularly as a biostimulant, fertiliser and/or soil conditioner.

### **Background**

- [0002] Seaweed has been used as a fertiliser and soil conditioner for many years.
- 10 Seaweed has been used in milled or finely divided form and in the form of a liquid extract. In general liquid extracts are preferred as they may be purchased as an aqueous concentrate, readily diluted to the required concentration and applied using agricultural spray equipment.
- [0003] Seaweed extracts have been shown to increase crop yields, resistance of plants
- 15 to frost, uptake of inorganic soil nutrients, resistance to environmental stress conditions, resistance to some pests such as red spider mite and aphids and to reduce fruit losses during storage. Seaweed extracts improve the root system of plants and increase their reproductive capacity, increasing the number of flowers per plant as well as the number of flowers per flowering season.
- 20 [0004] Seaweed species are grouped into red, green and brown seaweeds. The species used to produce extracts varies throughout the world. Most commercially available seaweed fertilisers, also called biostimulants, are made from brown seaweeds. Brown seaweeds are more prevalent in cold water regions of the world. In Australia, seaweed fertiliser products are mostly manufactured from alginate rich Bull Kelp or a blend with
- 25 other seaweed.
- [0005] Seaweed species vary in the amount of alginate present. The extraction process may also have a bearing on the amount and structure of alginate in the extract prepared from the seaweed. Alginates are important compounds in providing the soil conditioning properties of seaweed fertilisers and hence their presence in seaweed extracts is

regarded as beneficial.

[0006] The presence of alginate has however placed severe limitations on the use of other plant nutrients with seaweed extracts and in particular the use of calcium salts with seaweed extract.

5 [0007] Calcium is widely recognized for its important function in plants to increase nutrient uptake, build strong cell walls for sturdier plants and increase vitality. Maintaining high levels of bio-available calcium in soil is critical in achieving adequate levels of calcium uptake. Calcium plays a very important role in plant growth and nutrition, as well as in cell wall deposition. The primary roles of calcium as a soil  
10 amendment are to help maintain chemical balance in the soil, reduce soil salinity, and improve water penetration. Calcium also plays a critical metabolic role in carbohydrate removal and neutralizing cell acids. It is important to note that even though many soils contain high levels of insoluble calcium such as calcium carbonate, crops grown in these soils will often show symptoms of a calcium deficiency. Calcium also has roles in  
15 mitigating plant stresses such as cold and heat.

[0008] Balancing the requirement of high biostimulant efficacy with high levels of calcium and alginate content in the seaweed extract is a problem. When seaweed extract containing alginate is combined with calcium salts an insoluble gel is formed which produces residues which may interrupt, block and interfere with agricultural equipment  
20 such as spraying and irrigation equipment. The presence of the gel residue also gives rise to poor product stability which is exacerbated in cold weather conditions. Importantly, the unwanted gel residue is not reversible in nature. Consumers also find the presence of gels unattractive and there is a significant risk that a non-homogeneous composition will result in under-dosing of the components or a loss of a portion of the composition  
25 through failure to adequately disperse solids prior to dosing.

[0009] Previous attempts to address this problem have lead to the use of separate seaweed and calcium fertiliser components which are combined in very dilute compositions prior to spraying. The potential problems are minimised by contact of calcium and alginate species only in very dilute compositions, such as when

concentrates are diluted by, for example, dispersing one part by volume of concentrate in 200 parts by volume of water. This need to combine different materials adds to the cost of transport and handling of different compositions and also relies on appropriate dosing at the point of use. Others have obviated the problem by preparing solid particulate  
5 fertilisers which can be broadcast as solid fertilisers. This, however, loses the benefit of spray application which is a significant benefit of liquid seaweed extracts, furthermore when plants have a calcium deficiency, rapid uptake approaches such as foliar application mandate a liquid product. These types of deficiencies are inefficiently addressed via a slow release solid form product.

10 [0010] One example of a seaweed extract containing a calcium additive is disclosed in EP0884293 which teaches a combination of at least one natural or synthetic aminopurine derivative or an algal extract rich in such derivatives, and a source of calcium as an agent for stimulating the germination process and the development of the plant  
15 production structures. The sources of calcium may include limestone, calcium chloride and calcium salts of amino acids. This patent also teaches that aminopurine-rich extracts were obtained by extraction at pH 3 with sulphuric acid. The end-product (see Example 3 of EP0884293) is typically a solid.

[0011] A further example of a solid composition of a seaweed extract containing calcium is disclosed in EP 2268594. A water-soluble fertiliser suspension with calcium, soluble  
20 seaweed, and fulvic acid is processed into a solid powder form together with magnesium and phosphate in defined ranges.

[0012] The present inventors have examined the use of calcium chelation agents such as EDTA, fulvic acid, and amino acids in attempt to reduce the problem of gel formation on combining a liquid seaweed extract concentrate with a calcium source. Such strategies  
25 do not, however, lead to satisfactory liquid products when the liquid concentrate contains significant levels of alginate based on the weight of the concentrate composition.

[0013] It is desirable to have a one-pack liquid seaweed extract concentrate such as extract derived from one or more of Bull Kelp (*Durvillae potatorum*), Knotted Kelp

(*Ascophyllum nosodum*) and *Durvillaea* species *D. antarctica* that also contain calcium ions.

## Summary

[0014] There is provided an aqueous seaweed extract in the form of a solution

5 concentrate composition comprising:

- (a) at least 0.5 % by weight soluble alginate based on the weight of concentrate composition;
- (b) at least 0.1% calcium by weight based on the weight of concentrate composition;
- 10 (c) a calcium-chelating agent; and
- (d) a borate compound.

[0015] There is further provided a method of preparing an aqueous seaweed extract in the form of a solution concentrate as described above, the method comprising:

- 15 (a) extracting a seaweed to provide a seaweed extract comprising alginate in an amount of at least 0.5% w/w of the concentrate composition;
- (b) combining the extract with a calcium source in the presence of a calcium chelating agent and a borate compound to provide least 0.1% by weight calcium based of the weight of the concentrate composition.

[0016] There is thus further provided a method of fertilising plants comprising:

- 20 providing a liquid seaweed extract concentrate as herein described;  
optionally diluting the seaweed extract concentrate; and  
applying liquid seaweed extract to the plants or locus of the plants before, during  
or after planting.

[0017] The liquid seaweed extract may be applied in an amount to provide one or more  
25 benefit selected from the group consisting of enhanced plant growth, reduced plant stress and improved soil.

[0018] Throughout the description and claims of the specification, the word "comprise" and variations of the word, such as "comprising" and "comprises", is not intended to exclude other additives, components, integers or steps.

5 [0019] The term emulsion is used hereon to refer to a fine dispersion of minute droplets of one liquid in another in which it is not soluble. The composition may, for example, comprise an emulsified water immiscible phase. The term emulsion includes macro-emulsions and micro-emulsions.

10 [0020] The term "plant (or plants)" is a synonym of the term "crop" and is to be understood to include a plant of economic or aesthetic importance. The term "plant" as used herein includes all parts of a plant such as seed, leaves, roots, flowers, fruits, germinating seed, seedlings, cuttings, herbaceous vegetation as well as established woody plants including all belowground portions (such as the roots and tubers) and associated soil microbes, aboveground portions, plant endophytes and plant epiphytes.

15 [0021] The seaweed extract concentrate composition is a plant biostimulant. The term "biostimulant" in this context refers to materials, with the exception of synthetic nutrients and pesticides, which when applied to plants, have the capacity to modify physiological processes of plants in a way that provides potential benefits to growth, development and/or stress response.

### **Detailed Description**

20 [0022] The composition of the invention is in the form of a solution concentrate of seaweed extract and contains alginate and calcium ions despite the difficulties previously reported in obtaining such compositions due to the formation of insoluble gel formed from the reaction of soluble calcium ions and alginate. The composition is stabilised against formation of insoluble gels by the presence of a combination of calcium chelating agent and borate. While the composition comprises a solution concentrate of seaweed extract  
25 it may additionally comprise dispersions of one or more of solids and water immiscible liquids such as in the form of an emulsion, micellar solution or swollen micelles.

[0023] The concentrate composition and process involve a liquid seaweed extract. Suitable liquid seaweed extracts are commercially available and may be obtained commercially or prepared by extraction of suitable alginate containing seaweeds. Seaweed species are generally grouped into red, green and brown seaweeds. Although seaweed extracts from any of the general types containing alginates may be used the composition is particularly suited to brown seaweed and its extracts. Extracts of the brown seaweed (orders, families and genera in the class *Phaeophyceae*) are particularly suitable to the preparation of seaweed fertilisers and biostimulants and include *Acscophyllum*, *Durvillaea*, *Ecklonia*, *Laminaria*, *Lessonia*, *Macrocystis*, *Fucus* and *Sagassum*. Specific examples of brown seaweeds include Bull Kelp (*Durvillaea potatorum*), *Durvillaea* species *D. antarctica* and Knotted Kelp (*Ascophyllum nosodum*) which have high levels of alginate.

[0024] The solution concentrate may comprise a wide concentration range of dissolved solids depending on the seaweed extraction method, the type of seaweed and the materials including calcium salts, borate and any other materials which are added. In one set of embodiments the solution concentrate composition comprises at least 5% w/w dissolved solids and more preferable at least 10 % w/w dissolved solids by weight of the solution concentrate composition. The total dissolved solids is generally no more than 60% w/w, preferably no more than 50% w/w such as no more than 40% w/w or no more than 30% w/w.

[0025] In one set of embodiments the seaweed extract on a dry weight basis constitutes at least 20% w/w of the dry weight of the total solution concentrate with components not derived from the seaweed constituting the balance. More preferably the seaweed extract will comprise at least 50% w/w (dry weight) and still more preferably at least 80% w/w (dry weight) based on the dry weight of the solution concentrate composition. The dry weight amount of seaweed extract may preferably be no more than 99% w/w such as no more than 95% w/w seaweed extract dry weight based on the total dry weight of the solution concentrate.

[0026] Alginate, also called algin or alginic acid, is an anionic polysaccharide distributed

widely in the cell walls of seaweed, particularly brown seaweed. In extracted form it absorbs water quickly; it is capable of absorbing 200-300 times its own weight in water. Alginate is a linear copolymer with homopolymeric blocks of (1-4)-linked  $\beta$ -D-mannuronate (M) and its C-5 epimer  $\alpha$ -L-guluronate (G) residues, respectively,  
5 covalently linked together in different sequences or blocks.

[0027] The monomers can appear in homopolymeric blocks of consecutive G-residues (G-blocks), consecutive M-residues (M-blocks) or alternating M and G-residues (MG-blocks). The alginate is generally derived from the seaweed extract but if desired additional alginate may be added such as sodium alginate to supplement the seaweed  
10 extract content of alginate. The alginate (determined as alginic acid equivalent) is present in the composition in an amount of at least 0.5 % by weight based on the weight of the solution concentrate composition. In the case of extract from brown seaweed the content is generally at least 2% by weight of the weight of the concentrate composition, more preferably at least 5% w/w based on the weight of the concentrate composition.

15 Many of the available seaweed extracts comprise up to 25% by weight alginate based on the weight of the concentrate composition and generally we have found the composition to be particularly useful in agricultural applications with an alginate content (determined as alginic acid equivalent) in the range of from 8% to 25% by weight, more preferably 8% to 20% by weight, based on the weight of the concentrate composition.

20 [0028] There is provided, in one set of embodiments, an aqueous seaweed extract in the form of a solution concentrate composition comprising:

- (a) at least 0.5 % by weight soluble alginate based on the weight of concentrate composition;
- (b) at least 0.1% calcium by weight based on the weight of concentrate  
25 composition;
- (c) a calcium-chelating agent (preferably at least 0.1% by weight as calcium based on the weight of concentrate composition); and
- (d) a borate compound (0.01% by weight as boron based on the weight of concentrate composition).

[0029] The composition comprises dissolved calcium ions which may be derived from soluble calcium salts. The calcium may be added as a salt of the chelating agent or alternatively a calcium salt, particularly a water soluble calcium salt, may be used. The nature of the calcium salt used in adding calcium to the composition is not narrowly critical provided the salt is soluble and does not adversely interfere with the composition or cause plant injury. Typical examples of calcium salts include calcium nitrate (which also provides a nitrogen source), calcium chloride, calcium fulvate and calcium gluconate. The calcium salts with at least one of nitrate, chloride and gluconate counterions are particularly preferred. The calcium may be present in an amount of at least 0.1%, preferably from 0.1% to 5%, such as 0.2% to 4%, 0.3% to 4% or 0.4% to 3% by weight based on the weight of the concentrate composition. In one set of embodiments the calcium is present in an amount of no more than 3% such as no more than 1% by weight based on the weight of the concentrate composition.

[0030] The composition comprises borate. Borate compounds which may be used in the compositions of the present invention are boric acid and other agrochemically acceptable salts such as sodium borate (borax) and potassium borate. Borates include a range of boron-containing oxyanions. The term "borates" includes chemical compounds which contain borate anions. Larger borates are composed of trigonal planar  $\text{BO}_3$  or tetrahedral  $\text{BO}_4$  structural units, joined together via shared oxygen atoms and may be cyclic or linear in structure. Borates may be used or present in a range of hydrated forms and the process and composition may utilize any suitable form of at least one borate compound.

[0031] In one set of embodiments the borate compound comprises at least one selected from the group consisting of trialkyl borates, perborates, borax decahydrate, borax pentahydrate and boric acid.

[0032] The borate is present in an amount to reduce gel formation or preferably stabilize the composition against gel formation in the presence of the calcium cation. The borate concentration will thus depend on the concentration of calcium ion, alginate and the chelating agent. In one set of embodiments the borate is present in an amount in the

range of from 0.02 to 10% preferably 0.02 to 5% by weight (as boron equivalent) based on the weight of the concentrate composition and more preferably present in an amount in the range of from 0.02 to 0.5% by weight (as boron equivalent) based on the weight of the concentrate composition. In one set of embodiments the weight ratio of borate (as boron) to calcium is in the range of from 1:1 to 1:25 and more preferably from 1:3 to 1:15. In the specification and claims the reference to borate determined as boron or as boron equivalent means that the weight or weight percent of the borate component is reported in terms of only the boron content of the borate and not including the total borate which includes oxyanions and may include hydrates and or counterions.

[0033] Examples of forms of borax include anhydrous borax ( $\text{Na}_2\text{B}_4\text{O}_7$ ), borax pentahydrate ( $\text{Na}_2\text{B}_4\text{O}_7 \cdot 5\text{H}_2\text{O}$ ), Borax decahydrate ( $\text{Na}_2\text{B}_4\text{O}_7 \cdot 10\text{H}_2\text{O}$ ) and  $\text{Na}_2[\text{B}_4\text{O}_5(\text{OH})_4] \cdot 8\text{H}_2\text{O}$ . In one set of embodiments the borate compound is borax (as pentahydrate or decahydrate), and the concentration of borax is at least 0.5% by weight which corresponds to about 0.05% by weight as boron based on the weight of the concentrate composition.

[0034] The term chelating agent refers to an agent which provides chelation of calcium. According to the International Union of Pure and Applied Chemistry (IUPAC) chelation involves the formation or presence of two or more separate coordinate bonds between a polydentate (multiple bonded) ligand and a single central atom which in the present context is calcium. Usually these ligands are organic compounds, and are also called chelants, chelators or sequestering agents. A wide range of chelating agents for calcium are known in the art and a skilled person will be able to select a suitable chelating agent without undue experimentation. The calcium chelating agent content will depend in part on the calcium concentration and the borate concentration. In one set of embodiments the amount of calcium chelating agent is present in an amount in the range of from 0.1% to 8% by weight based on the weight of the concentrate composition. Preferably the calcium chelating agent is present in an amount in the range of from 0.5% to 5% w/w of the concentrate composition. In one set of embodiments the molar ratio of chelating agent : calcium is in the range of from 12:1 to 1:4 and preferably 4:1 to 1:4.

[0035] Specific examples of calcium chelating agent include gluconodeltalactone (GDL), gluconic acid, ethylenediaminetetraacetic acid (EDTA), carboxy-functional sugars, amino acids, fulvic acid, humic acids, ethylene glycol tetraacetic acid (EGTA), cyclohexyldiaminetetraacetic acid (CDTA), diethylenetriamine pentaacetic acid (DTPA), nitritotriacetic acid (NTA), N-(hydroxyethyl)-ethylenediaminetriacetic acid (HEDTA), (N-(1,2-dicarboxyethyl)-D,L-aspartic acid (IDS), DS (polyaspartic acid), EDDS (ethylenediamine-N,N'-disuccinic acid), GLDA (N,N-bis(carboxymethyl)glutamic acid) and MGDA (methylglycinediacetic acid). The chelating agent may be added in the form of the acid or water soluble salt. It may be used in the form of the calcium salt with the chelated calcium providing at least portion of the calcium content of the concentrate composition. In a particularly preferred set of embodiments the calcium chelating agent is GDL and the weight ratio of GDL to calcium is at least 2, preferably at least 2.5 such as in the range 2.5 to 4.

[0036] The composition is an aqueous solution of the seaweed extract. In one set of embodiments the aqueous solution concentrate comprises at least 40% w/w water, preferably at least 60 % w/w water and more preferably at least 70% w/w water. The composition may comprise up to 95% w/w water but in the preferred embodiments the composition comprises up to 90% w/w water such as up to 85% w/w water.

[0037] In one set of embodiments the seaweed extract solution concentrate composition comprises:

- (a) alginate in an amount in the range of from 5% to 25%, preferably 3% to 20%, more preferably 5% to 20%, by weight of the weight of the concentrate composition;
- (b) calcium in an amount of from 0.1% to 5%, preferably 0.1% to 4%, more preferably 0.2% to 2%, by weight of the weight of the concentrate composition;
- (c) borate in an amount in the range of from 0.02 to 10%, preferably 0.02% to 5%, more preferably 0.02 to 0.5%, still more preferably 0.03% to 2%, by weight (as boron equivalent) of the weight of the concentrate composition;

- (d) calcium chelating agent in the range of from 0.1% to 8%, preferably 0.1% to 5%, more preferably 0.3 to 5%, by weight of the weight of the concentrate composition; and
- (e) water in an amount of from 60% to 95% w/w of the concentrate composition, preferably 70% to 90% w/w of the concentrate composition.

[0038] In a preferred aspect of this set embodiments the molar ratio of chelating agent: calcium is in the range of from 12:1 to 1:12 more preferably 4:1 to 1:4.

[0039] The composition may be prepared with a wide range of pH values depending on the method used for preparing the extract and/or pH adjustment following extraction. In one set of embodiments the pH of the solution concentrate is in the range of from 7 to 12.5 and preferably in this embodiment the pH is from 9.0 to 11.5. The pH the liquid composition may be provided by an alkalising agent such as an alkanolamine or a metal hydroxide.

[0040] The solution concentrate may comprise further materials such as one or more selected from the group consisting of plant nutrients, pesticides, plant safeners, plant growth regulators, plant defence elicitors, soil enhancers, microbial nutrients, and beneficial biologicals, or parts, such as microbes.

[0041] In one set of embodiments the composition comprises additional fertiliser components (which may be soluble and/or insoluble) such as at least one selected from the group of additional nitrogen, phosphorus and potassium. Examples of such supplements may include urea, nitrates, potassium salts, magnesium salts, selenium salts and other micronutrients.

[0042] The composition of the invention may include a source of phosphorus. Seaweed concentrates comprising additional phosphorus are frequently difficult to provide because of the formation of insoluble calcium phosphate species. Useful phosphorous compounds which may be included in compositions of the invention include, but are not limited to, phosphates (as trivalent anion), monohydrogen phosphates (as divalent anion), dihydrogen phosphates (as monovalent anion), phosphoric acid, hypophosphite,

and phosphate adducts such as urea phosphate. The total phosphate (determined as phosphate and not including counter ion) is preferably in the range of from 0.5% to 10% by weight, preferably from 1% to 10% and more preferably 3.5% to 7% by weight, based on the weight of the seaweed extract.

5 [0043] Accordingly in one set of embodiments, the concentrate compositions further comprise one or more phosphorous compounds, such as phosphorus compounds selected from the group consisting of phosphates, dihydrogen phosphates, monohydrogen phosphates, dihydrogen phosphates, phosphoric acid, hypophosphates, polyphosphates, and phosphates adducts such as urea phosphates. Particularly  
10 preferred phosphates include monohydrogen phosphates, hypophosphates, mono ammonium phosphate and mixtures thereof, still more preferably the phosphorus compound is mono ammonium phosphate. In one preferred set of embodiments the concentrate composition comprises at least one phosphorous compound and the pH is in the range 9 to 12.5, preferably pH10 to pH11.

15 [0044] The solution concentrate may comprise active agents selected from the group consisting of herbicides, insecticides, fungicides and bio-control agents including microbial or, microbial derived bio-control agents. The aqueous seaweed solution concentrate may comprise additional materials in one or more form selected from the group consisting of dispersed solids, dispersed liquids emulsions and micellar moieties  
20 Micellar moieties may be swollen.

[0045] Examples of water soluble herbicides include herbicidal acid salts including as synthetic auxin salts such as salts of one or more of 2,4-D, dicamba, MCPA, triclopyr and clopyralid, organophosphorus salt herbicides such as salts of glyphosate and glufosinate, aryphenoxypropionates (FOPS) salts, sulfonyleureas salts such as salts of sulfosulfuron  
25 and imidazolinones. Such herbicides may be formulated as water soluble compositions particularly using the water soluble herbicidal salts.

[0046] Examples of water insoluble materials which may be dispersed include water insoluble esters of acid herbicides such esters of synthetic auxin herbicides, biological

materials such as Bt toxins and bacterial cells or spores such as Bt cells or spores, triazine herbicides, imidacloprid insecticide.

[0047] In one set of embodiments, biological plant protection agents are mixed with the liquid seaweed concentrates. Biological plant protection agents are materials that

5   comprise proteins and/or spores derived from biological (rather than synthetic chemical) processes. Biological plant protection agents include but are not limited to one or more of the set comprising CryA, CryB, VIP and bacillus proteins and/or spores. Bacillus spores and/or toxins may be derived from *Bacillus thuringiensis* (Bt) bacteria, such as Bt subspecies *Kurstaki* strain ABTS-351, Bt subspecies *Kurstaki* strain HD1, or from  
10   *Bacillus firmus* bacteria or other bacteria. Bacillus spores and/or toxins may be obtained from commercially available suspension concentrate or dry flowable formulations of biological plant protection agents such as Dipel SC or Dipel DF. For example Dipel DF can be dispersed directly into the liquid seaweed concentrate of the invention, or may be dispersed in water and the dispersion added to the liquid seaweed concentrate of the  
15   invention. Dipel SC (which carried in an oily phase) may also be directly or indirectly added into the liquid seaweed concentrate of the invention. In one embodiment a composition comprising a biological plant protection agent is dispersed in the liquid seaweed concentrate by combining from 2 to 20% by weight biological plant protection agent such as Dipel DF with liquid seaweed concentrate.

20   [0048] Other biological materials that can be coformulated with the liquid seaweed extract include viruses and virus-derived materials, fungi and fungal materials, nematodes and proteins. Examples of viral materials include baculoviruses that infect and kill the larvae of moths and other larvae. In one preferment the baculovirus is a nucleopolyhedrovirus (NPV) of *helioverpa* larvae such as is contained in the commercial products Gemstar  
25   (distributed by Sipcam and by Certis) and Vivus Gold (distributed by AG Biotech Australia). Some of these biological materials are sold commercially as aqueous suspensions, and such suspensions can be added to pre-formed liquid seaweed concentrate of the invention.

[0049] The liquid seaweed concentrate may be combined with endophytes, including bacterial or fungal endophytes. Endophytes live within a plant for at least part of their life cycles without causing disease, and may act to (i) prevent colonisation of the plant by pathogenic organisms or (ii) inhibit competitor plants. A large variety of endophyte/plant synergies have been identified. Fungal endophytes may be from the phylum Ascomycota (e.g. orders Hypocreales and Hymenochaetales of the Pyrenomycete class). Commercially available endophytes including the following ryegrass endophytes: AR37, AR1, Endo5. Companies that provide endophytes on a commercial basis include PGG Wrightson Seeds (Australia) and Agricom. Endophytic bacteria may include bacteria that promote biological nitrogen fixation (without nodule formation) such as *Herbaspirillum seropedicae*. Other diazotrophic bacteria include *Gluconacetobacter diazotrophicus*, *H. rubrisubalbicans*, *Azospirillum amazonense* and *Burkholderia tropica*. Inoculant formulations of endophytes may be provided in polymer carriers such as carboxymethyl cellulose (CMC) solutions, and polymeric alginates contained in liquid seaweed concentrates of the invention may contribute to the stability and viability of endophytes.

[0050] In one set of embodiments the liquid seaweed concentrate composition comprising the biological plant protection agent in an amount such as from 2 to 20% by weight is applied to plants to control target insects without harming bees or beneficial insects.

[0051] The liquid seaweed solution concentrate may be applied by spraying in the concentrate form if desired, but will generally be diluted with water prior to application to plants or the locus of plants before, during or after planting. Water is used as a carrier for the solution concentrate to enable different dilutions fit for purpose and for different application methods. The solution concentrate may be used by dilution with water generally at a rate in the range of from 1 part by volume concentrate to from 4 to 1000 parts by volume of water, such as 20 to 1000 parts or 50 to 500 parts, by volume of water. In one embodiment the concentrate may be tank mixed with other agrochemicals such as one or more selected from the group consisting of fertilisers, pesticides (herbicides, fungicides and insecticides) and plant growth regulators.

[0052] The concentrate composition may also be diluted with water at a rate in the range of from 1 part solution concentrate to from 4 to 20 parts, preferably 4 to 8 parts by volume of water.

[0053] In one set of embodiments the aqueous seaweed extract in the form of a solution concentrate is prepared by a method comprising:

- (a) extracting a seaweed to provide a seaweed extract concentrate comprising alginate in an amount of at least 0.5% by weight based on the weight of the extract;
- (b) combining the extract with a calcium source in the presence of a calcium chelating agent and a borate compound to provide a concentrate composition as hereinbefore described containing calcium in an amount of least 0.2% by weight based on the weight of the concentrate composition.

[0054] In one set of embodiments, the liquid composition of the invention is formulated by a process involving the following steps in sequence:

- (i) charging concentrated high-alginate seaweed extract liquid to a first vessel,
- (ii) making up a calcium chelate pre-mix solution in a second vessel, and
- (iii) slowly adding the contents of the second vessel to the first vessel, wherein the contents of the first vessel are preferably agitated while the addition takes place. The borate component will preferably be premixed with the seaweed extract in the first vessel or the chelating composition in the second vessel.

[0055] In one set of embodiments, the first vessel is charged with 80-95 parts by weight high-alginate seaweed extract having 10 – 25% soluble solids, and the second vessel contains 10 parts by weight of calcium chelate pre-mix which is added to the first vessel after charging with high-alginate seaweed extract.

[0056] The high-alginate seaweed extracts can be made, purchased from commercial sources or may be prepared using any of a range of methods known to the art. These include the following:

- US 2036922 (filed 1935, Clark and Green, "Alginic acid and process of making same") provides an improved process for making alginates of high viscosity from kelp. The improvement is to reduce hydrolytic break-down of alginic acid in processing. The process involves first leaching with cold fresh water and hydrochloric acid, chopping and then hot digesting with sufficient sodium carbonate in solution to provide a final diluted pulp of pH in the range 9 – 11. The digestion time is 40 minutes and heating is provided by the admission of live steam. The rough pulp is further comminuted to finely divide it, and pulp dilution and filtration then takes place.
- Characterisation and QC methods discussed in the literature include: G to M ratio, molecular weight, molecular weight distribution, molecular weight stability, gel strength in various conditions of gel formation, viscosity in standard conditions, alginic acid weight percent.
- US 1814981 (filed 1927, Curtis and Walsh, "Process of preparing alginic acid and compounds thereof ") provides an important early description of alginate production which provided a pure and stable product (note that alginic acid hydrolysis occurs in acid and in hot conditions). Raw kelp is harvested by under-water cutting and loaded into pre-leaching tanks. Pre-leaching is done at ordinary temperatures and has the objective of removing adherent sea salt. Kelp is transferred to leaching tanks which operate at 140 deg. F. Aged liquor from the leaching tanks can be used for re-leaching. After pre-leaching, kelp is drained and roughly chopped up, and in the leach tanks an aqueous solution of sodium carbonate is added. Addition of steam is used to heat the pulp. The rough pulp is ground so that the sodium carbonate has more area on which to act. The pulp issuing from the grinding mill is diluted at about 140 deg. F with 5 times its volume of warm water, and the diluted pulp is boiled by the injection of live steam. The creamy pulp formed is pumped to storage tanks and filtered to remove cellular debris, e.g. using rotary suction filters.
- CN 101445414 (filed 2008, "Formulation of seaweed gel soil conditioner and processing method thereof ") teaches taking freshly harvested kelp and (i) washing

with twice the weight of water with a 4-hr soak, (ii) size reduction in a colloid mill, (iii) placing in a -10 deg. C freezer and cooling at the rate of 2 - 4 deg. C per hour, thawing the complex to provide a paste and (iv) adding fertiliser salts and antioxidants and (v) packing.

- 5     •     WO 2012123422 (filed 2012, Susilo, "Method for processing seaweed") teaches a process with (i) removal of seaweed from the ocean, (ii) treatment with aqueous potassium hydroxide at a temperature below 50 deg. C for not longer than 6 hours, (iii) separation of solid and liquid ingredients, (iv) processing to powder form (by spray drying or freeze drying). An optional process feature is adding potassium
- 10     chloride to the potassium hydroxide liquor in the second step above.
- US 2829773 (filed 1955, Saddington, "Method of clarifying alginic liquor") describes methodology for removing cellular debris such as a continuous 2-stage process including flotation and sedimentation steps. Haze particles are removed using smaller-than-expected filter aid particles of vesiculated perlite mineral.
- 15     [0057] It is generally preferred that the aqueous solution concentrate comprise a seaweed extracted under alkaline conditions such as a pH of at least 9 to such as 9 to 12.5.

[0058] In another set of embodiments there is provided a method of fertilising plants comprising:

- providing a liquid seaweed extract concentrate as herein described;
- 20     •     optionally diluting the seaweed extract concentrate; and
- applying the liquid seaweed extract to the plants or locus of the plants before, during or after planting in an amount to provide one or more benefit selected from the group consisting of enhanced plant growth, reduced plant stress and improved soil.
- 25     [0059] The composition may be used in fertilising plants by application to plants and or locus of plants. This includes application to soil, or other growth medium, before, during or after planting. Where the composition comprises herbicides, selective herbicides may

be used or the composition may be applied before or during planting. Selective herbicides may be used to allow foliar spray application to a crop to control weeds within the crop. In one embodiment the composition is applied to seeds or locus of seeds at seeding. The liquid seaweed extract may, for example, be applied by soaking seed, seed coating or by application to soil proximate seed. Application to soil may take place before, during or after placement of seed. The composition is preferably applied in an amount to provide enhanced seed and or plant growth and or soil properties.

[0060] The composition may for example be applied during crop seeding, for example by application from a mechanical seeder.

[0061] The amount of composition used in each application will depend on the period between application of the composition (optionally following dilution) and the concentration of the concentrate. In one set of embodiments the rate of concentrate use is at least 1L concentrate per hectare, preferably at least 5L concentrate per hectare.

[0062] The concentrate composition may be used in a wide range of horticultural applications and application rates. For instance in one specific example the composition is added at a rate of about ten litres of concentrate per hectare added to as much spray water as the farmer uses for 1 hectare. In one set of embodiments the composition is applied in the range of from 1 to 10 applications per growing season. The number of applications per growing season may be chosen together with the rate of application to provide an economical increase in production of commercial crops. For example in one set of embodiments the concentrate is applied at a rate of 10 litres per hectare with about 4 applications per season. Alternatively a user may apply the concentrate at a lower rate of for example 5L per hectare more frequently (e.g. 8 times per season).

[0063] Higher rates (10L/ha) are particularly useful for plants coping with stress. Further, it is possible to prime plants with an initial application of the composition at lesser rates, followed by a higher rate of application.

[0064] Application methods include foliar application, irrigation, spraying, soaking, aerial, underground irrigation, chemigation, fertigation and open furrow application.

[0065] The concentrate composition may be used to improve the growth and/or condition of a wide range of plant species. Examples of typical plants on which the composition may be used include, without limitation, grapes including wine and table grapes, nuts such as almonds and macadamia, potatoes, citrus, tropical crops such as mangos, avocados, sugarcane, apples and bananas, vegetables such as carrots, onions, broccoli and garlic, fruiting plants such as tomatoes, strawberries, blueberries, other berries, cereals & broad acre crops (such as wheat, barley, canola, rice and cotton), turf and pastures plants, legume plants such as beans, soybean and clover and greenhouse crops.

[0066] The invention will now be described with reference to the following examples.

[0067] It is to be understood that the examples are provided by way of illustration of the invention and that they are in no way limiting to the scope of the invention.

## 15 **EXAMPLES**

[0068] The Examples of the invention are discussed with reference to the attached drawings.

### **Brief Description of the Drawings**

[0069] In the drawings:

20 [0070] **Figure 1** is a graph of the variation in gel particle size formed within the borate containing composition of the invention with the ratio of gluconodeltalactone (GDL)/ calcium ion. The upper plot shows mean particle size and the lower plot shows the median particle size.

[0071] **Comparative Examples 1 to 7**

[0072] These comparative examples examine the use of calcium chelating agents (in the absence of borate salts) in an attempt to stabilise an aqueous liquid seaweed extract concentrate.

[0073] The seaweed extract sold under the SEASOL<sup>®</sup> brand was purchased from a commercial hardware store in Australia and had the specification shown in Table 1. The soluble solids content was increased from 15.5% by weight to 18 % by weight by evaporation (this concentrate is a liquid with 82% w/w water content). The 18 % w/w solids content was a solution without any precipitate.

[0074] The compositions of Comparative Examples 1 to 7 were prepared in accordance with the process in Table 2 and the resulting composition was examined for gel and or precipitate formation with the results shown in Table 2.

[0075] The chelating agents alone failed to provide the stability suitable for storage transport and handling of the seaweed extract concentrate with soluble calcium moieties.

[0076] The examples refer to a number of seaweed concentrates including the SEASOL<sup>®</sup> seaweed concentrate product.

**[0077] Composition of SEASOL<sup>®</sup> Seaweed Extract (®– trademark of SEASOL International Pty Ltd)**

[0078] **Table 1**

Component/feature	Amount	Units
pH	10.5	
Total solids	15.5	%w/w
Specific gravity	1.1	Grams /ml
appearance	Dark brown liquid, seaweed odour	
filtration	150	microns

Element		
nitrogen	0.2%	w/v
phosphorous	0.02%	w/v
potassium	3.7%	w/v
boron	15	mg/L
calcium	458	mg/L
cobalt	<0.5	mg/L
copper	<0.5	mg/L
iron	115	mg/L
magnesium	972	mg/L
manganese	2	mg/L
molybdenum	<0.5	mg/L
selenium	<0.5	mg/L
silicon	56	mg/L
sodium	6820	mg/L
sulfur	2,574	mg/L
zinc	5	

[0079] **Table 2**[0080] **Comparative Example (CE) Composition and results**

CE Number	formulation	Calcium chelating Moiety	results
1	Start with SEASOL <sup>®</sup> 18% soluble solids. Add 4% calcium nitrate, then add 1.5% tetrasodium EDTA	EDTA	Large gel particles formed
2	Make complex (with 1.5 parts tetrasodium EDTA, 4.5 parts calcium nitrate, 1 part fulvic acid in 5 parts water. Add 10 parts complex to	EDTA, fulvic acid	Layer formed at bottom of container

	(90 parts) SEASOL® (18% soluble solids)		
3	Make complex (with 8 parts HEDTA(40% soln in water) and 5 parts calcium nitrate. Add 2 parts complex to 90 parts SEASOL® (18% soluble solids).	Hydroxyethylenediamine triacetic acid (HEDTA)	Large gel particles formed
4	Add 10 parts calcium fulvate powder to 90 parts SEASOL® (18% soluble solids)	Fulvic acid	Large gel particles formed
5	Into 84 parts SEASOL® (18% soluble solids) add 1 part calcium hydroxide and 15 parts glucono delta lactone.	glucono delta lactone	Crystal precipitate
6	Make complex with 5 parts calcium nitrate, 3 parts gluconodelta lactone, 1.9 parts monoethanolamine and 4.5 parts water. Add 10 parts complex to 90 parts SEASOL® (18% solublesolids).	glucono delta lactone	Large gel particles formed
7	Add 5 parts fulvic acid powder plus 6 parts calcium nitrate to 90 parts SEASOL® 18% solids	Fulvic acid	Phase separation, Product not uniform

[0081] **Example 1 (preparation of a seaweed extract concentrate composition according to the invention)**

[0082] The seaweed extract concentrate composition of Example 1 is prepared by making a calcium chelate pre-mix (see table entry below designated part1:calcium chelate pre-mix) and incorporating the calcium chelate pre-mix into SEASOL® liquid extract followed by mixing and pH adjustment (see Table 3 below). The calcium chelate pre-mix comprises 1) a Source of calcium, 2) a chelating moiety for calcium, 3) a boron source and 4) a pH additive.

[0083] The seaweed extract concentrate composition of Example 1 is prepared by the steps shown in Table 3 with the components and amounts specified.

[0084] **Table 3**

<b>calcium containing liquid seaweed extract of the invention</b>	<b>Amount Required (kg)</b>
Ensure tank is clean and valve is closed.	
Seaweed extract with Soluble solids 18% (Part 2)	90kg
Start Stirrer	
Slowly add calcium chelate pre-mix (Part 1)	13.2 kg
Mix for 10 minutes	
Test pH then add	
Potassium Hydroxide	1kg
Add till pH above 10.9 to 11.4	
Mix for 5 minutes after each addition	
Total	104.2kg

<b>Part 1: Calcium Chelate Pre-mix</b>	<b>Amount Required (kg)</b>
Ensure tank is clean and valve is closed.	
Water (Heat to 40°C)	60kg
Start Stirrer	
Calcium Nitrate tetrahydrate	47kg
Glucono Delta Lactone	29kg
Sodium tetraborate decahydrate (Borax)	11kg
Monethanolamine	23kg
Measure pH =7.5 to 9	
Total	170kg

[0085] The final composition made using the above procedure had the following composition shown in Table 4:

5 [0086] **Table 4**

Item	%w/w
SEASOL <sup>®</sup> (18% soluble solids)	86.37
Added water	4.47
Calcium nitrate tetrahydrate	3.50
Glucono delta lactone	2.16
Sodium tetraborate decahydrate (borax)	0.82
monoethanolamine	1.71
Potassium hydroxide	0.96
Total	99.99

[0087] The density of the final composition was 1.14 kg per litre.

[0088] The concentration of glucono delta lactone in the final composition was 2.16 %w/w,  
5 and at density 1.14 kg/L this corresponds to 2.46 %w/v.

[0089] The concentration of sodium borate (as sodium tetraborate decahydrate,  $\text{Na}_2\text{B}_4\text{O}_7 \cdot 10\text{H}_2\text{O}$ , molecular weight 381.4) in the final composition was 0.82%w/w and at formulation density 1.14 kg/L this corresponds to 0.93% w/v. The concentration in terms of boron was  $4 \times 10.8 / 381.4 \times 0.82 = 0.093\%$  w/w and at formulation density 1.14 kg/L this  
10 corresponds to 0.11% w/v.

[0090] The concentration of calcium nitrate tetrahydrate ( $\text{Ca}(\text{NO}_3)_2 \cdot 4\text{H}_2\text{O}$ , molecular weight 236.1) in the final composition was 3.50% w/w and at formulation density 1.14 kg/L this corresponds to about 4% w/v as calcium nitrate tetrahydrate. The concentration in terms of calcium was  $40 / 236.1 \times 3.5 = 0.59\%$  w/w and at formulation density 1.14 kg/L  
15 this corresponds to 0.68% w/v.

[0091] The concentration of alginate in the final composition was about 10% w/w.

[0092] **Example 2 (preparation of a liquid calcium containing seaweed extract concentrate composition according to the invention)**

[0093] The seaweed extract concentrate of Example 2 is prepared using the procedure and amounts of components shown in Table 5 below.

[0094] **Table 5**

<b>Calcium containing liquid seaweed concentrate of the invention</b>	<b>Amount Required (kg)</b>
Ensure tank is clean and valve is closed.	
SEASOL <sup>®</sup> (Soluble solids 18%) (Part 2)	90kg
Start Stirrer	
Slowly add calcium chelate pre-mix (PART 1)	13.7kg
Mix for 10 minutes	
Test pH then add	
Potassium Hydroxide	1kg
Add till pH above 10.9 to 11.4	
Mix for 5 minutes after each addition	
Total	104.7kg

<b>Part 1: Calcium Chelate Premix</b>	<b>Amount Required (kg)</b>
Ensure tank is clean and valve is closed.	
Water (Heat to 40°C)	60kg
Start Stirrer	
Calcium Nitrate tetrahydrate	50kg
Glucono Delta Lactone	29kg
Sodium Borate (Borax, tetrasodium borate decahydrate)	11kg
Potassium Hydroxide	6kg
Measure pH =7.5 to 9	
Total	156kg

[0095] The final composition of Example 2 made using the above procedure had the following composition shown in Table 6:

[0096] **Table 6**

Item	%w/w
SEASOL® (18% soluble solids)	85.96
Added water	5.03
Calcium nitrate tetrahydrate	4.19
Glucono Delta lactone	2.43
Sodium borate	0.92
Potassium hydroxide	1.5
Total	100.03

5 [0097] The density of the final composition was 1.14 kg per litre.

[0098] The concentration of glucono delta lactone in the final composition was 2.43% w/w, and at density 1.14 kg/L this corresponds to 2.77% w/v.

[0099] The concentration of sodium borate (as sodium tetraborate decahydrate,  $\text{Na}_2\text{B}_4\text{O}_7 \cdot 10\text{H}_2\text{O}$ , molec weight 381.4) in the final composition was 0.92% w/w and at  
 10 composition density 1.14 kg/L this corresponds to 1.05% w/v. The concentration in terms of boron was  $4 \times 10.8 / 381.4 \times 0.92 = 0.10\%$  w/w and at formulation density 1.14 kg/L this corresponds to 0.12% w/v.

[0100] The concentration of calcium nitrate tetrahydrate ( $\text{Ca}(\text{NO}_3)_2 \cdot 4\text{H}_2\text{O}$ , molec weight 236.1) in the final composition was 4.19% w/w and at formulation density density 1.14  
 15 kg/L this corresponds to 4.78% w/v. The concentration in terms of calcium was  $40 / 236.1 \times 4.19 = 0.71\%$  w/w and at formulation density 1.14 kg/L this corresponds to 0.81%w/v.

[0101] The concentration of alginate in the final composition was 10% w/w.

[0102] **Example 3 (preparation of a calcium containing liquid seaweed extract concentrate composition according to the invention)**

[0103] The seaweed extract composition of Example 3, in which sodium gluconate is the calcium chelator, is prepared by the general process described in Example 1 and  
 5 detailed in Table 7 using the components in the amounts specified.

[0104] **Table 7**

<b>Calcium containing liquid seaweed concentrate of the invention</b>	<b>Amount Required (kg)</b>
Ensure tank is clean and valve is closed.	
Seaweed extract with Soluble solids 18% ( Part 2)	90kg
Start Stirrer	
Slowly add calcium chelate pre-mix (PART 1)	13.4 kg
Mix for 10 minutes	
Test pH then add	
Potassium Hydroxide	1kg
Add till pH above 10.9 to 11.4	
Mix for 5 minutes after each addition	
Total	104.4kg

<b>Part 1: Calcium Chelate Pre-mix</b>	<b>Amount Required (kg)</b>
Ensure tank is clean and valve is closed.	
Water (Heat to 40°C)	60kg
Start Stirrer	
Calcium Nitrate tetrahydrate	47kg
Sodium gluconate	29kg
Sodium tetraborate decahydrate (Borax)	10.9kg
Monethanolamine	3.5kg
Measure pH =7.5 to 9	
Total	150.4kg

[0105] **Example 4 (evaluation of composition described in Example 2)**

[0106] The size and number of gel particles was estimated by using a Dino-Lite Handheld Digital Camera inserted in a Microscope in conjunction with DinoCapture 2 software from AnMo Electronics Corporation. A sample of the seaweed concentrate composition of Example 2 was placed on a microscope slide under a cover slip and the Dino digital was fitted into microscope. After image focussing, a photo was taken and captured on the software (which carried out further analysis). The sieve test was conducted by running 100 ml of concentrate through a sieve and drying and weighing the residue. The results are shown in Table 8.

[0107] **Protocols for particle size, average no of particles, sieve test**

[0108] **Table 8**

	Typical gel particle size (mm)	Av no of gel particles per sq 0.1mmx0.1mm	Sieve test 53 microns.
Example 2	0.009	10	0.16%

[0109] The Comparative Examples 1 to 7 contained very significant gelation with relatively large particles (or phase separation) which would render the product difficult or impossible to apply with farm spray equipment whereas the composition of the invention contained insignificant levels of particulates of size which would not interfere with normal spray application. Also the presence of significant particles reduces the bioavailability of the calcium alginate component which is bound in gel form.

[0110] **Example 5**

[0111] This example demonstrates the invention using a variety of six distinct borate compounds. These six distinct borate compounds were:

1. Trimethyl borate - CAS 121-43-7, (purchased from Sigma-Aldrich Australia), purity > 99%, chemical formula  $B(OCH_3)_3$ , mole weight 103.91 g.

2. Triisopropyl borate - CAS 5419-55-6 (purchased from Sigma-Aldrich Australia), purity > 98%, chemical formula  $B((CH_3)_2CHO)_3$ , mole weight 188.07 g.
3. Sodium Perborate tetrahydrate CAS 10486-00-7 (purchased from Acros Chemicals, Australia), dimer (divalent) anion  $B_2O_4(OH)_4$ , 2 boron atoms joined by 2 peroxo bridges, average composition  $NaBO_3 \cdot 4H_2O$ .
4. Boric acid, chemical composition  $B(OH)_3$ , mole weight 61.8g.
5. Borax, (sodium borate, sodium tetraborate, disodium tetraborate, sodium tetraborate decahydrate), chemical formula  $Na_2B_4O_7 \cdot 10H_2O$ , molar mass 381.38 (decahydrate) 201.22 (anhydrate).
6. Borax (sodium tetraborate pentahydrate, chemical formula  $Na_2B_4O_7 \cdot 5H_2O$ .

[0112] Six different calcium chelate pre-mixes were made by mixing the components shown in Table 9:

[0113] **Table 9**

Part 1: Calcium chelate pre-mix	parts
Ensure tank is clean and valve is closed.	
Water (Heat to 40°C)	60
Start Stirrer	
Calcium nitrate tetrahydrate salt	47
Glucono Delta Lactone	29
Borate compound	11
Monethanolamine	23
Measure pH =7.5 to 9	
Total	170

- [0114] The six calcium chelate pre-mixes were designated trimethyl borate pre-mix, triisopropyl borate pre-mix, sodium perborate tetrahydrate pre-mix, boric acid pre-mix, borax decahydrate pre-mix and borax pentahydrate pre-mix.

[0115] Six different formulations of the invention were made by adding 14 parts of calcium chelate pre-mix (6 variations as discussed above) to 86 parts seaweed extract (SEASOL<sup>®</sup>, 18% solids).

[0116] Composition details for these six formulations are provided in table 10 below:

5 [0117] **Table 10**

Procedure	Trimethyl borate	Triiso-propyl borate	Sodium perborate tetrahydrate	Boric acid	Borax decahydrate	Borax pentahydrate
	Parts by weight	Parts by weight	Parts by weight	Parts by weight	Parts by weight	Parts by weight
(i) Add SEASOL <sup>®</sup> , 18% solids	86	86	86	86	86	86
(ii) Add calcium chelate pre-mix	14	14	14	14	14	14
Percentage of final boron contain Compound content (Percentage Boron equivalent)	0.74 (0.08 as boron)	0.74 (0.04 as boron)	0.74 (0.10 as boron)	0.95 (0.17 as boron)	0.99 (0.11 as boron)	0.73 (0.108 as boron)

[0118] The formulations shown in Table 10 were examined as follows: The size and number of gel particles was estimated using a Dino-Lite Handheld Digital Camera inserted in a Microscope in conjunction with DinoCapture 2 software from AnMo Electronics Corporation (see Example 4). Results are shown in Table 11.

[0119] **Table 11**

parameter	Trimethyl borate formulation	Triisopropyl borate formulation	Sodium perborate tetrahydrate formulation	Boric acid formulation	Borax Decahydrate formulation	Borax pentahydrate formulation
Average gel particle size (mm)	0.009	0.014	0.0114	0.0443	0.0058	0.0045
Standard deviation	0.0056	0.0118	0.0088	0.0237	0.0046	0.002
Average no. of gel particles per sq 0.1mmx0.1mm	120	188	192	184	78	48

[0120] **Discussion**

[0121] Based on gel particle size (smaller the better) and particle number (fewer the better), boron as borax (pentahydrate form) gave the best formulation and boric acid gave the worst formulation. Other boron moieties were significantly better than boric acid.

[0122] **Example 6**

[0123] The following example explores the effect of increasing the calcium concentration (as calcium nitrate tetrahydrate) in formulations of the invention. Calcium-induced precipitation is the key problem addressed by the instant invention, and increasing calcium represents increasing likelihood of instability (associated with the formation of large and/or numerous gel particles).

[0124] Four calcium chelate pre-mixes C1, C2, C3 and C4 with varying quantities of calcium (added as calcium nitrate tetrahydrate) were prepared as provided in Table 12.

[0125] **Table 12**

component	C1	C2	C3	C4
	Parts by weight	Parts by weight	Parts by weight	Parts by weight
water	75	50	40	36
Calcium nitrate tetrahydrate	35	60	70	80
Gluconolactone	29	29	29	23
Borax (pentahydrate)	8.74	8.74	8.74	8.74
Potassium hydroxide				
monoethanolamine	10	10	10	10
Total	157.74	157.74	157.74	157.74
Percent (rounded) calcium when calcium chelate (14 parts) is added to SEASOL® (18% solids) 86 parts	0.5%	0.9%	1.1%	1.2%

[0126] Four formulations of the invention were made by taking 86 parts SEASOL® (18% solids) and stirring in 14 parts of the above four calcium chelate pre-mixes C1, C2, C3, C4.. These 4 formulations of the invention were designated FC1, FC2, FC3 and FC4 respectively.

[0127] The formulations FC1, FC2, FC3 and FC4 were examined as follows: The size and number of gel particles was estimated using a Dino-Lite Handheld Digital Camera inserted in a Microscope in conjunction with DinoCapture 2 software from AnMo Electronics Corporation (see Example 4). Results are shown in Table 13.

[0128] **Table 13**

parameter	FC1	FC2	FC3	FC4
Average gel particle size (mm)	0.0054	0.011	0.052	0.044
Standard deviation	0.0051	0.005	0.044	0.038

[0129] **Discussion**

[0130] The above formulations were passed through a 53 micron sieve, and it was observed that the quantity of residue on the sieve was greater for FC3 than for FC1 and FC2, and the quantity of residue for FC4 was significantly greater than for FC3. Gel particle size tends to increase with increasing calcium. The size increase is greatest when the calcium content is between 0.9% and 1.1%. This preferred calcium concentration range level will vary depending on borate loading.

[0131] Note that in the above experiment, the best (small) gel particle sizes are encountered when the ratio of gluconodeltalactone (GDL) to calcium (as calcium nitrate tetrahydrate is greater than around 2.5, and preferably greater than 3. These results are substantiated in Table 14:

- ◆ Calcium ion concentration based on the fact that in the calcium nitrate used, calcium comprises 17% of weight.
- ◆ So if there are 35 parts calcium nitrate in a total formulation weight of 157.74 parts (see Table 14 below for formulation with 0.52% calcium, rounded to 0.5%), the percent calcium in the formulation is  $3.77\% = (35 \times 0.17 / 157.74)$ .
- ◆ If there are 29 parts GDL in 157.74 parts total formulation, the percent GDL in the formulation is  $18.38 = 29 \times 100 / 157.74$
- ◆ In this scenario, the ratio of GDL to calcium is  $18.38 / 3.77 = 4.9$  (approx).

[0132] **Table 14**

Formulation character	F0.5% Cal	F0.9% Cal	F1.1%Cal	F1.2% Cal
Water	75	50	40	36
Calcium Nitrate	35	60	70	80
Gluc Delta lactone	29	29	29	23
Monethanolamine	10	10	10	10
Sodium Borate Pentahydrate	8.74	8.74	8.74	8.74
Total mass	157.74	157.74	157.74	157.74

Calcium Ion Concentration %	3.77	6.5	7.54	8.62
GDL mass %	18.38	18.38	18.38	14.58
Ratio GDL/Ca	4.9	2.8	2.4	1.7
Particle Size Mean	0.005	0.011	0.052	0.044
Median size	0.0038	0.0101	0.036	0.0331

[0133] The variation of particle size with the ratio of gluconodeltalactone (GDL) to calcium ion is shown in Figure 1.

[0134] The above formulations FC1, FC2, FC3, FC4 were re-made by withholding borate components from the above-noted calcium chelate pre-mixes, and by adding the withheld borate material to the SEASOL<sup>®</sup> (18% solids). This addition took place before the final step where calcium chelate pre-mix (14 parts) was added to SEASOL<sup>®</sup> (18% solids) (84 parts). It was found that the variation of gel particle size with calcium was substantially the same.

#### [0135] **Example 7**

[0136] In this example various calcium compounds were examined for use as a source of calcium ions used in the compositions of the invention. The water solubility of calcium salts examined is provided in Table 15.

[0137] **Table 15**

Calcium salt	% Calcium	Mean molecular weight	Solubility in water
Calcium Chloride dihydrate	37%	147.02	>80 g /100ml
Calcium hydroxide	54%	Ca(OH) <sub>2</sub> 74.093 g/mol	0.173 g/100 mL (20 °C)
Calcium citrate tribasic	24 %	498.4334 g/mol (anhydrous)	0.085 g/100 mL (18 °C) 0.095 g/100 mL (25
Calcium gluconate	9%	448.4g/mol	Needs heat to dissolve in water 3.5% in cold water
Calcium Fulvate	7%	Polymer	30% soluble in water
Calcium Carbonate	40%	100 g/mol	Almost insoluble in water
Calcium nitrate tetrahydrate	17%	236.15g/mol	>80 g /100ml

[0138] Four calcium chelate pre-mixes were made by utilising the four water soluble calcium salts: calcium nitrate, calcium chloride, calcium gluconate and calcium fulvate.

- 5 These calcium chelate pre-mixes were designated nitrate, chloride, gluconate and fulvate pre-mixes respectively. The components used in preparing compositions of the four calcium chelate pre-mixes are shown in Table 16.1 to 16.4.:

[0139] **Table 16.1**

Nitrate Calcium chelate pre-mix	parts
Ensure tank is clean and valve is closed.	
Water (Heat to 40°C)	60
Start Stirrer	
Calcium Nitrate tetrahydrate	47
Glucono Delta Lactone	29
Sodium tetraborate decahydrate (Borax)	11

Monethanolamine	23
Measure pH =7.5 to 9	
Total	170

[0140] **Table 16.2**

<b>Chloride Calcium chelate pre-mix</b>	parts
Ensure tank is clean and valve is closed.	
Water (Heat to 40°C)	60
Start Stirrer	
Calcium chloride	50
Glucono Delta Lactone	29
Sodium tetraborate pentahydrate (Borax)	8.74
Monethanolamine	10
Measure pH =7.5 to 9	
Total	157.74

[0141] **Table 16.3**

<b>Gluconate Calcium chelate pre-mix</b>	parts
Ensure tank is clean and valve is closed.	
Water (Heat to 40°C)	60
Start Stirrer	
Calcium gluconate	79
Sodium tetraborate pentahydrate (Borax)	10
Monethanolamine	6
water	10
Measure pH =7.5 to 9	
Total	165

[0142] **Table 16.4**

<b>Fulvate Calcium chelate pre-mix</b>	parts
Ensure tank is clean and valve is closed.	
Water (Heat to 40°C)	50
Start Stirrer	
Calcium nitrate	60
Potassium Fulvate	30
Sodium tetraborate pentahydrate (Borax)	8.75
Monethanolamine	5
Measure pH =7.5 to 9	
Total	153.75

[0143] The nitrate, chloride and fulvate calcium chelate pre-mixes were added at 10 parts to 90 parts of SEASOL (18% solids), and these formulations shown in Table 17 were examined.

[0144] The size of gel particles was estimated using a Dino-Lite Handheld Digital Camera inserted in a Microscope in conjunction with DinoCapture 2 software from AnMo Electronics Corporation (see Example 4). Results were as follows:

[0145] **Table 17**

parameter	nitrate	fulvate	chloride
Mean size (mm)	0.011	0.025	0.016
Std (mm)	0.011	0.028	0.015

[0146] The calcium gluconate complex was heated for a period of about one hour at 50°C to it render soluble and when this complex (10 parts) was added to SEASOL<sup>®</sup> (18% solids) 90 parts, a clear solution was found with very few gel particles.

[0147] **Example 8**

[0148] The following phosphate salts were included in a liquid seaweed plus calcium formulation of the invention:

5 Monoammonium phosphate (MAP), chemical formula  $\text{NH}_4\text{H}_2\text{PO}_4$ , mole weight 115g, water solubility (20 deg C) 404 g/L, phosphorous 27%.

Monopotassium phosphate (MKP), chemical formula  $\text{KH}_2\text{PO}_4$ , mole weight 136.1g, water solubility (20deg C) 226g/L, phosphorous 23%.

10 Sodium triphosphate (STP equals STPP for sodium tripolyphosphate), chemical formula  $\text{Na}_5\text{P}_3\text{O}_{10}$ , mole weight 367.9g, water solubility (25 deg C) 145 g/L, phosphorous 25%.

[0149] Four different formulations of the invention were made by the following 4-step procedure:

- 15 (i) Seaweed extract (18% solids),  
(ii) calcium chelate pre-mix,  
(iii) phosphate moiety, and  
(iv) optional pH adjustment.

20 [0150] The four formulations were designated MAP (low pH), MAP (high pH), MKP, and STP.

[0151] The SEASOL<sup>®</sup> 18% solids composition was obtained by evaporation of the product shown in Table 1 to obtain 18%w/w solids content as described in Comparative Example 1.

[0152] The calcium chelate premix used was as shown in Table 3 (Part 1).

25 [0153] Formulation details are provided in Table 18 below.

[0154] **Table 18**

Procedure	MAP (low pH)	MAP ( high pH)	MKP	STP
	Parts by weight	Parts by weight	Parts by weight	Parts by weight
(i) Add Seasol, 18% solids	81.8	81.8	80.4	82.0
(ii) Add calcium complex	14	14	14	14
(iii) add phosphorous moiety				
MAP	4.25	4.25	-	-
MKP	-	-	5.64	-
STP	-	-	-	4
Phosphorous (%)	0.9%	0.9%	1.2%	1.0%
(iv) pH adjustment	No pH adjustment, final pH equals 8.08, final composition comprises 4.25 parts MAP	pH adjusted up by KOH, final pH equals 10.56 final composition comprises 4.25 parts MAP	pH adjusted up by KOH, final pH equals 10.5 final composition comprises 5.64 parts MKP	No pH adjustment, final pH equals 10.5, final composition comprises 4 parts STP

[0155] The formulations shown in Table 18 were examined as follows: The size and number of gel particles was estimated using a Dino-Lite Handheld Digital Camera inserted in a Microscope in conjunction with DinoCapture 2 software from AnMo Electronics Corporation (see Example 4). Results were as follows:

[0156] **Table 19**

parameter	MAP low pH	MAP high pH	MKP	STP
Average gel particle size (mm)	.023	.011	.0099	.054
Standard deviation	.009	.006	.0088	.033
Average no. of gel particles per sq. 0.1mmx0.1mm	144	46	48 unstable with crystals	108

[0157] Based on low gel particle size and high formulation stability, the optimal phosphorous-containing formulation was achieved by adding MAP to the SEASOL<sup>®</sup> 18% plus calcium chelate pre-mix liquors, and by taking the final pH to the range 9-11.5.

[0158] **Example 9**

5 [0159] This Example demonstrated preparation of compositions of the invention with a variety of sources of seaweed.

[0160] The following seaweed extracts were obtained and/or formulated:

10 [0161] SWE1 –Seaweed extract powder (manufacturer Acadian Seaplants) was obtained and dissolved at 18% solids in water, with pH adjustment to 10.5 with potassium hydroxide.

[0162] SWE2 – Seaweed liquid concentrate (Stimplex<sup>®</sup>, a liquid, 19.5% solids) Ascophyllium nodosum seaweed concentrate sold by Acadian. This was obtained and taken to pH 10.5 with potassium hydroxide.

15 [0163] Formulations according to the invention (FSWE1 and FSWE2 respectively) were made by mixing the nitrate calcium chelate premix of Table 3 (Part 1)(10 parts) to seaweed extracts SWE1 and SWE2 respectively.

[0164] The size of gel particles was estimated using a Dino-Lite Handheld Digital Camera inserted in a Microscope in conjunction with DinoCapture 2 software from AnMo Electronics Corporation (see Example 4). Results are shown in Table 20:

20

[0165] **Table 20**

<i>parameter</i>	<i>FSWE1</i>	<i>FSWE2</i>
Mean size (mm)	0.0069	0.0051
Std (mm)	0.0063	0.0047

[0166] **Example 10**

[0167] This example examines the effect of increasing the borate concentration (as borax pentahydrate) in formulations of the invention.

[0168] Four calcium chelate pre-mixes with different quantities of borax (as pentahydrate)  
5 were prepared as provided in Table 21.

[0169] **Table 21**

component	<b>3 parts borate in calcium chelate pre- mix</b>	<b>5 parts borate in calcium chelate pre- mix</b>	<b>7 parts borate in calcium chelate pre- mix</b>	<b>10.9 parts borate in calcium chelate pre-mix</b>
	Parts by weight	Parts by weight	Parts by weight	Parts by weight
water	60	60	60	60
Calcium nitrate tetrahydrate	50	50	50	50
Gluconolactone	29	29	29	29
Borax (pentahydrate )	3	5	7	10.9
Potassium hydroxide	0	0	0	0
monoethanolamine	7	7	7	6

[0170] Four formulations of the invention were made by taking 86 parts SEASOL® (18%  
10 solids) and stirring in 14 parts of the above four calcium chelate pre-mixes. These four formulations of the invention were designated F3, F5, F7 and F10.9 respectively.

[0171] The formulations F3, F5, F7 and F10.9 were examined as follows: The size and number of gel particles was estimated using a Dino-Lite Handheld Digital Camera inserted in a Microscope in conjunction with DinoCapture 2 software from AnMo  
15 Electronics Corporation (see Example 4). Results are shown in Table 22:

[0172] **Table 22**

parameter	F3	F5	F7	F10.9
Average gel particle size (mm)	0.068	0.021	0.0086	0.007
Standard deviation	0.063	0.016	0.0036	0.006

[0173] **Discussion**

5 [0174] There is a consistent trend to lower gel particle sizes when higher borate levels are used.

[0175] **Example 11**

[0176] In this example the chelating agent potassium fulvate was included by mixing the following components in the parts by weight specified in Table 23:

[0177] **Table 23**

Fulvic Calcium Chelate pre-mix	parts
Ensure tank is clean and valve is closed.	
Water (Heat to 40°C)	70
Start Stirrer	
Calcium nitrate tetrahydrate	40
Potassium fulvate	30
Sodium perborate decahydrate	10
Monethanolamine	5
Measure pH =7.5 to 9	

[0178] 14 Parts Fulvic calcium chelate pre-mix mixed with 86 parts Seasol (18% solids), and potassium hydroxide was used to achieve a pH of between 10.5 and 11.0. The final formulation was designated FFA.

5

[0179] The size of gel particles was estimated using a Dino-Lite Handheld Digital Camera inserted in a Microscope in conjunction with DinoCapture 2 software from AnMo Electronics Corporation (see Example 4). Results were as follows:

parameter	FFA
Mean size (mm)	0.011
Std (mm)	0.011

10

[0180] **Example 12 Bioefficacy data:**

[0181] Bioefficacy was measured in terms of root growth when (i) seedlings were immersed in Commercial SEASOL<sup>®</sup> concentrate of the composition of Table 1 (1/400 dilution in water), and compared with (ii) seedlings immersed in Calcium containing liquid seaweed concentrate of the invention (in Table 10 using the Borax pentahydrate formulation and process) (1/400 dilution in water).

15

[0182] The greenhouse conditions for the comparison were: 22-25 deg. C at 60% relative humidity and using Capsicum seedlings with trimmed roots and grown for a 14 day duration at which point the roots were harvested, dried and their weight measured.

[0183] The following percent root weight gains were found when Calcium containing liquid seaweed concentrate of the invention was added to water according to the above protocol: capsicum seedlings in the treatment with Calcium containing liquid seaweed concentrate of the invention had 48% greater dry root weight relative to capsicum seedlings treated with commercial SEASOL<sup>®</sup> concentrate.

20

25

<b>Treatment</b>	<b>Dried weight of roots (g) average</b>
SEASOL®	0.074
SEASOL® + Ca	0.110

- 5 [0184] The increase in the dried weight of roots in the presence of the stable calcium liquid seaweed concentrate of the invention represents a significant improvement in the enhanced growth.

**Claims**

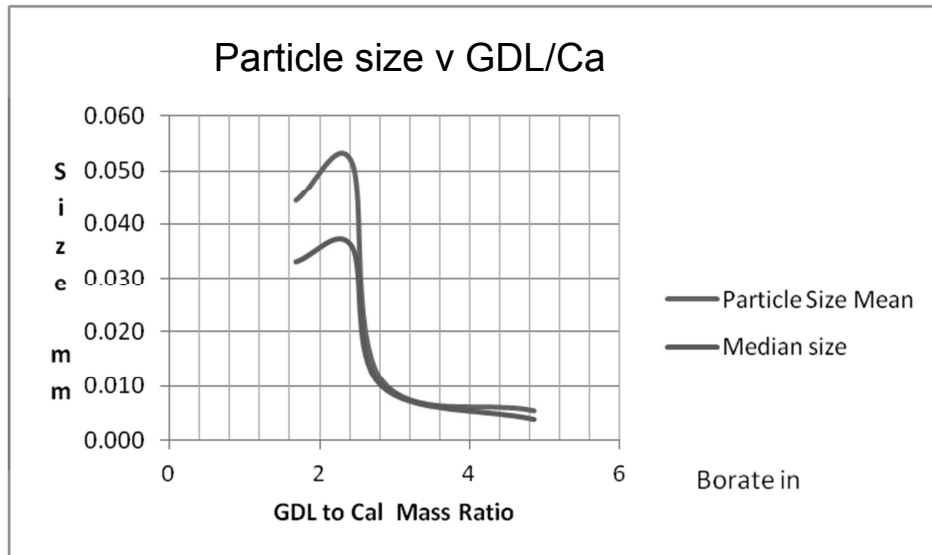
1. There is provided an aqueous seaweed extract in the form of a solution concentrate composition comprising:
  - (a) at least 0.5 % by weight soluble alginate based on the weight of concentrate composition;
  - (b) at least 0.1% calcium by weight based on the weight of concentrate composition;
  - (c) a calcium-chelating agent; and
  - (d) a borate compound.
2. An aqueous seaweed extract according to claim 1, wherein the soluble alginate is present in an amount of at least 2 % by weight of the concentrate composition.
3. An aqueous seaweed extract according to any one of the previous claims, wherein the borate (as boron equivalent) is present in an amount in the range of from 0.02 to 5 % by weight based on the weight of the concentrate composition.
4. An aqueous seaweed extract according to any one of the previous claims, wherein the weight ratio of borate (as boron equivalent) to calcium is in the range of from 1:1 to 1:25.
5. An aqueous seaweed extract according to any one of the previous claims, wherein calcium chelating agent is present in an amount in the range of from 0.1 % to 8 % by weight based on the weight of the concentrate composition.
6. An aqueous seaweed extract according to any one of the previous claims, wherein the molar ratio of chelating agent : calcium is in the range of from 12:1 to 1:4.
7. An aqueous seaweed extract according to any one of the previous claims, comprising at least 60 % w/w water.

8. An aqueous seaweed extract according to any one of the previous claims, wherein the composition comprises no more than 95 % w/w water.
9. An aqueous seaweed extract according to any one of the previous claims, wherein the seaweed extract solution concentrate composition comprises:
  - (a) alginate in an amount in the range of from 5 % to 25 % by weight of the weight of the concentrate composition;
  - (b) calcium in an amount of from 0.1 % to 5 % by weight of the weight of the concentrate composition;
  - (c) borate (as boron equivalent) in an amount in the range of from 0.02% to 5% by weight of the weight of the concentrate composition;
  - (d) calcium chelating agent in the range of from 0.1 % to 8 % by weight of the concentrate composition; and
  - (e) water in an amount of from 60 % to 95 % w/w of the concentrate composition.
10. An aqueous seaweed extract according to any one of the previous claims wherein the borate is present in an amount of from 0.02 to 0.5% (as boron equivalent) by weight of the concentrate composition.
11. An aqueous seaweed extract according to any one of the previous claims wherein the calcium is in the form of a calcium salt comprising at least one selected from the group consisting of calcium nitrate, calcium chloride, calcium fulvate and calcium gluconate.
12. An aqueous seaweed extract according to any one of the previous claims, wherein the pH of the solution concentrate is in the range of from 7 to 12.5.
13. An aqueous seaweed extract according to any one of the previous claims comprising one or more further materials selected from the group consisting of plant nutrients, pesticides, plant safeners, plant defence elicitors, soil enhancers, microbial nutrients, and beneficial biologicals, or parts, such as microbes.

14. An aqueous seaweed extract according to any one of the previous claims, comprising one or more further materials dispersed or emulsified in the solution concentrate or present in micelles.
15. An aqueous seaweed extract according to any one of the previous claim wherein the composition comprises one or more additional fertiliser components selected from the group consisting of additional nitrogen, phosphorus and potassium components.
16. An aqueous seaweed extract according to any one of the previous claim, wherein the composition comprises one or more plant protection agents selected from the group consisting of herbicides, insecticides and fungicides.
17. An aqueous seaweed extract according to claim 16 wherein the plant protection agent is a biological plant protection agent selected from the group consisting of *Bacillus thuringiensis*; *Bacillus firmus*; the proteins, spores and toxins of at least one of *Bacillus thuringiensis* and *Bacillus firmus*; baculovirus; and endophytes.
18. An aqueous seaweed extract according to any one of the previous claim, wherein the calcium is present in an amount of no more than 3% by weight of the weight of the concentrate composition.
19. An aqueous seaweed extract according to any one of the previous claims, comprising the chelating agent gluconodeltalactone (GDL) and wherein the weight ratio of GDL to calcium ion is at least 2 preferably at least 2.4.
20. An aqueous seaweed extract according to any one of the previous claims, wherein the borate compound comprises at least one selected from the group consisting of trialkyl borates, perborates, borax decahydrate, borax pentahydrate and boric acid.
21. An aqueous seaweed extract according to any one of the previous claims, wherein the boron compound is hydrated borax.

22. An aqueous seaweed extract according to any one of the previous claims, wherein the composition further comprises one or more phosphorous compounds.
23. An aqueous seaweed extract according to claim 22 wherein the phosphorus compound comprise at least one selected from the group consisting of monohydrogen phosphates, hypophosphates, monoammonium phosphate and diammonium phosphate.
24. An aqueous seaweed extract according to claim 22 or claim 23 wherein the composition comprising one or more phosphorous compounds has a pH is in the range of pH7 to pH12.5.
25. A method of preparing an aqueous seaweed extract in the form of a solution concentrate, the method comprising:
  - (a) extracting a seaweed to provide a seaweed extract comprising alginate in an amount of at least 0.5% w/w, of the concentrate composition;
  - (b) combining the extract with a calcium source in the presence of a calcium chelating agent and a borate compound to provide least 0.1% w/w calcium to provide a composition according to any one of the previous claims.
26. A method according to claim 25, wherein
  - (a) the alginate is present in the extract alginate in an amount in the range of from 5 % to 25 % by weight of the weight of the concentrate composition;
  - (b) the calcium source is a water soluble calcium salt which provides calcium in an amount of from 0.1 % to 5 % by weight of the weight of the composition;
  - (c) the borate (determined as boron equivalent) is present in an amount in the range of from 0.02 % to 5% by weight of the weight of the concentrate composition;
  - (d) the calcium chelating agent is present in the range of from 0.1 % to 8% by weight of the weight of the concentrate composition; and
  - (e) water in an amount of from 60 % to 95 % w/w of the solution concentrate.

27. A method according to claim 25 and claim 26 wherein the calcium source is a calcium salt comprising at least one selected from the group consisting of calcium nitrate, calcium chloride, calcium fulvate and calcium gluconate.
28. A method of enhancing plant growth comprising:
  - (a) providing a liquid seaweed extract concentrate composition according to any one of claims 1 to 22;
  - (b) optionally diluting the seaweed extract concentrate composition; and
  - (c) applying the liquid seaweed extract to the plants or locus of the plants before, during or after planting in an amount to provide one or more benefit selected from the group consisting of improved plant vigour, enhanced plant growth, reduced plant stress and improved soil.
29. A method according to claim 28, wherein the seaweed extract concentrate composition is diluted with water at a rate in the range of from 1 part by volume concentrate to from 4 to 1000 parts by volume of water.

**Figure 1**