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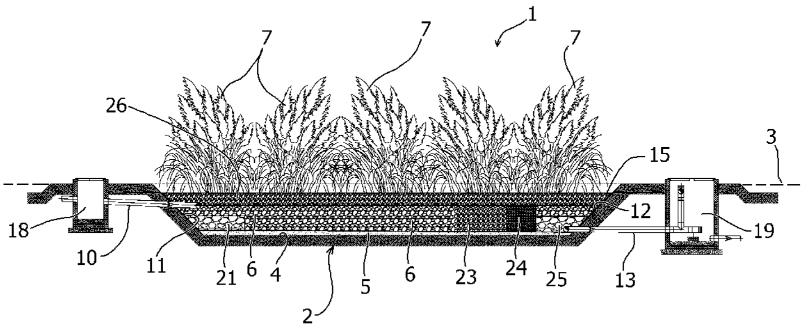

(57) **Abstract**: A biotechnical phytodepuration system (1) comprises a filtering bed (6) constituted by inert porous materials and vegetable species (7) planted on the filtering bed (6), where the filtering bed is inoculated with a consortium of microorganisms comprising : a) at least one symbiont fungus belonging to the genus Glomus spp. or Gigaspora spp., b) at least one saprophytic fungus belonging to the genus Trichoderma spp., and c) at least one bacterium of the rhizosphere selected among the genera Bacillus spp., Pseudomonas spp. and Actinomycetales spp.

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Biotechnological phytodepuration system

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FIELD OF THE INVENTION

The present description concerns a phytodepuration system for polluted water. In particular, the present description concerns a system for the biotechnological phytodepuration of polluted water/polluted waste from agriculture, livestock and aquaculture activities.

TECHNICAL BACKGROUND

Artificial phytodepuration systems are substantially similar to those established spontaneously in marshes and wetlands, in which the biological actions and interactions between hydrophilic plants and the resident microbial communities provide spontaneous auto-depuration of the water. During the gradual flow from the inlet to the outlet of these systems there is a continuous process of filtration and oxidation of suspended organic solids and a decrease in the dissolved salts that can be used by the roots of the aquatic plants present.

Artificial phytodepuration systems (also known as constructed wetlands) are essentially constituted of impermeable tanks containing a filtering bed of porous inert material (such as sand and gravel) on which hydrophilic plants are planted and through which the polluted water is made to flow.

Artificial phytodepuration systems provide more control with respect to natural systems, in that they allow more flexibility in defining the filtering bed, the type of vegetation and the hydraulic pathways. Moreover, such systems provide additional advantages such as site choice and flexibility in the choice of dimensions and geometry, as well as control of flow rates, retention times and pollutant loads, allowing their measurement at the inlet and outlet and the determination of their efficiency.

In particular, the use in filtration beds of materials with diverse physical characteristics (for example, porosity) and of chemically "active" materials (for example zeolites), instead of chemically inert materials such as gravel and sand, and the choice of specific vegetable species, can provide favourable results with decidedly lower depurative surfaces, independently of other input parameters (flow rate, waste characteristics, etc.).

Artificial phytodepuration systems are divided into a) horizontal submerged flow systems (SFS-h or *Subsurface Flow System - horizontal*) and b) vertical submerged flow systems (SFS-v or *Subsurface Flow System - vertical)*.

Artificial phytodepuration systems are known in the art, for example for the documents EP-A-0 390 265, US-A-5 951 866 and DE-A-196 25 328, where such systems envision that the filtration bed is constituted of inert porous materials, such as gravel and clinoptilolite (EP-A-0 390 265), pumice, lava material and/or synthetic or ceramic porous material (US-A-5 951 866) or a mixture of lava material and zeolites (DE-A-196 25 328). In addition, the document EP-A-1 414 756 envisions that the filtration bed consist exclusively of zeolites.

Until now, agro-food wastes for example from the wine, cheese, olive oil, livestock and aquaculture industries are treated using a) methanization procedures, wherein the organic material is stored under anaerobic conditions at a controlled temperature (about 37°C), b) procedures of spreading onto agricultural lands, which are limited by local laws, among other things, c) evaporative procedures that separate the solid from the liquid fractions and where the solid fraction must then be subjected to additional treatments, d) composting procedures. However, these system do not allow the efficient treatment of such wastes because of the particular composition of the wastes themselves and of the operative conditions of the treatment systems themselves.

Oenological wastes, for example generated from the cleaning required for proper winery hygiene, are constituted essentially of wash water that contains solids and liquids that characterise the waste as polluting. Various categories of polluting agents are contained in such waste:

- the residues of by-products transported with the wash: stems, seeds, skins, sediments, lees, tartrate incrustations,
- leakage of products such as: must, wine, both during the various washings and in case of accidental losses,
- products used for the treatment of wine: fining agents, diatomaceous earth, etc., as well as
- products for the washing and disinfection of equipment and surfaces.

The same components of must and wine are also found in oenological waste: sugars, alcohol, esters, glycerol, organic acids (tartaric, malic, lactic, acetic) and phenolic substances.

All of these substances have high chemical oxygen demand (COD) and, with the exception of phenolics, have a good level of biodegradability.

The average chemico-physical characteristics of oenological wastes are reported in table 1, wherein SST stands for total suspended solids.

Table 1.

OENOLOGICAL WASTES	
pH	4.1-6, occasionally 10-13 in the disincrusting phase
SST	1-2 g/l
COD	3-20 g of O_2/l
Volume produced	30-500 litres per hectolitre of wine produced, 40%-60% of which during the grape harvest

Industrial milk processing for the production of butter and cheeses also generates considerable quantities of polluting wastes, the principal component of which is whey.

Whey, while being substantially free of toxic agents or inhibitors of bacteria, cannot be released directly into bodies of water due to its elevated organic content and its treatment by means of classical biological depuration systems is difficult and expensive. The principal components of whey from the processing of cow's milk are indicated in table 2.

Table 2.

WHEY	
pH	4.9-5.8
SST	6.5%
COD	30,000-70,000 mg of O_2/l
Lactose	4-4.6%
Protein	0.65-0.75%
Whey proteins	0.55-0.65%
Casein	0.05-0.1%
Fats	0.06%
Salts	0.6-0.7%
Organic acids	0.15-0.20%
Ash	0.5%
Vitamins	12 mg/l

The composition of wastes generated in the processing of milk for the production of ricotta cheese and butter, called ricotta whey “scotta” and buttermilk, respectively, are reported in tables 3 and 4.

Table 3.

RICOTTA WHEY "SCOTTA"	
pH	5.6-6.2
COD	10,000-20,000 mg of O_2/l
Total protein	0.10-0.15%
Fat	0.15-0.30%
Lactose	4-4.6%
Salts	0.9-1.1%
Organic acids	0.20-0.25%

Table 4.

BUTTERMILK	
pH	4.5-5.1
Total protein	3.3-3.5%
Fat	0.5-0.8%
Lactose	4-4.6%
Salts	0.7-0.9%
Organic acids	0.15-0.20%
Whey proteins	0.6-0.7%
Casein	2.7-2.8%

The stretching water, which constitutes the residual waste from the stretching of the curds during the production of mozzarella, contains a high level of sodium chloride and a notable content of citric and lactic acids and is characterised by a high COD of about 2,000-50,000 mg of O_2/l.

The waste produced during olive oil extraction are known as vegetation water and pomace.

The composition of so-called vegetation water in function of the method of extraction used is reported in table 5, where BOD_5 indicates the biological oxygen demand.

Table 5.

VEGETATION WATER		
	Continuous process by centrifugation	Discontinuous process by pressing
pH	5.1-5.8	4.7-5.5

COD	55,000-208,000 mg of O_2/l	30,000-80,000 mg of O_2/l
BOD_5	20,000-90,000 mg of O_2/l	17,000-42,000 mg of O_2/l
Polyphenols	1.2-2.4	0.3-0.8
Water	79.85-86.4%	90.4-96.5%
Organic compounds	7.22-18.3%	2.6-8%
Fatty substances	0.02-1%	0.5-2.3%
Nitrogenous substances	1.2-2.4%	0.17-0.4%
Sugars	2-8%	0.5-2.6%
Organic acids	0.5-1.5%	trace
polyalcohols	1-1.5%	0.9-1.4%
Pectins, mucilages, tannins	1.3-1.7%	0.23-0.5%
P_2O_5	0.21%	0.06%
SO_3-SiO_2-FeO-MgO	0.09%	0.02%
K_2O	0.71%	0.19%
Na_2O	0.1%	0.03%

Regarding the pomace, besides further extraction of oil, it is normally used as fuel due to its considerable caloric value (4,000 kCal/Kg), except when a high percentage of humidity in the dry fraction renders such use inconvenient, in which case it is necessary to treat the pomace as polluting waste.

The pomace cannot be placed into the normal system of superficial water because being the by-product of the production of oil by grinding and pressing olives, it is constituted of seeds, skins and all the other solid parts of the drupe. It consists of 95% water and biomass and about 5% oil, which is recovered by means of extraction with solvents after drying.

The composition of livestock effluents is variable and depends on the species raised, on the breeding techniques, on the method of harvesting and handling the manure.

As a function of the type of housing and management, the effluents can be:

slurries: manure more or less diluted with wash water, water for transport or from leakage of the water system and drinking water overflow.

b) solid material: effluents in solid form that are amenable to the formation of piles.

The chemical characteristics of manures and other shovelable materials produced by diverse species are reported - in general terms - in table 6.

Table 6.

	Dry material (% t.q.)	Volatile solids (% SS)	N (Kg/t t.q.)	P (Kg/t t.q.)	K (Kg/t t.q.)
Bovine manure	20-30	75-85	3-7	0.4-1.7	3.3-8.3
Porcine manure	25	70	4.7	1.8	4.5
Porcine manure (deep bed)	42	78	8.2	9.5	12
Ovine manure	22-40	70-75	6-11	0.7-1.3	12-18

In view of the peculiarity of wastes produced by agro-food activities, such as wineries, dairy and olive oil processing, and by livestock and aquaculture the known phytodepuration systems do not provide efficacious treatment of such wastes, which - when released from the phytodepuration system - still contain polluting substances frequently with a high chemical oxygen demand (COD) and a high biological oxygen demand (BOD_5) and therefore cannot be released into superficial waters, because they would cause a reduction in the dissolved oxygen in the receiving water, which would be detrimental to the fish fauna, among others.

SUMMARY OF THE INVENTION

Taking these premises into consideration, the need is therefore felt for improved solutions that allow efficacious phytodepuration of polluted water and, for example, of wastes from wineries, dairy and olive oil processing, livestock and aquaculture.

The object of the present description is that of providing such improved solutions.

According to the invention, the above-said object is obtained by means of the solution specifically recalled in the attached claims, which constitute an integral part of the present description.

One embodiment of the present invention concerns a biotechnological phytodepuration system for polluted waste that comprises a tank containing a filtration bed constituted by a porous material and vegetable species planted on the filtration bed, an inlet for introducing the polluted waste into the tank and an outlet for the release of the depurated waste from the tank, in which the filtration bed is inoculated with a consortium of bacteria and fungi, where such consortium permits the reduction of the polluting contents as well as the COD and/or BOD_5 of the treated waste and also limits possible odours caused by aerobic and anaerobic fermentations.

A preferred embodiment of the present invention contemplates a biotechnological artificial phytodepuration system of the horizontal submerged flow (SFS-h) type comprising a filtration bed, preferably of high porosity, comprising at least one from: clay, pyroclastic rock, limestone rock, sepiolite, diatomaceous earth, or mixtures thereof, hydrophilic vegetable species planted on the filtering bed and a consortium of bacteria and fungi periodically inoculated in the filtering bed, wherein such consortium reduces the polluting contents as well as the COD and/or the BOD_5 of the treated waste and also limits possible odours caused by the aerobic and anaerobic fermentations.

In a particular embodiment the biotechnical phytodepuration system envisions that the consortium of bacteria and fungi inoculated in the filtering bed comprises:

a) at least one symbiont fungus belonging to the genus Glomus spp. or Gigaspora spp.,

b) at least one saprophytic fungus belonging to the genus Trichoderma spp., and

c) at least one bacterium of the rhizosphere selected among the genera *Bacillus* spp., *Pseudomonas* spp. and *Actinomycetales* spp.

Additionally, the bacterial and/or fungal consortium inoculated in the filtering bed can advantageously comprise:

- at least one biosurfactant fungus (or yeast) selected among the genera *Acinetobacter* spp., *Pichia* spp., *Torulopsis spp., Candida spp., Saccharomyces spp., Schizonella spp., Ustilago spp. and/or*

- at least one biosurfactant bacterium selected among the genera *Agrobacterium* spp., *Serratia* spp., *Flavobacterium* spp., *Mycobacterium spp., Nocardia spp., Corynebacterium spp., Rhodococcus spp., Arthrobacter spp., Thiobacillus spp., Gluconobacter spp., Aspergillus spp., Alcanivorax spp.*

Additionally, the bacterial and/or fungal consortium inoculated in the filtering bed can advantageously comprise a natural consortium of protozoa of the genus *Vorticella* spp., *Opercularia* spp., *Colpidium* sp., *Satrophilus* sp. and *Cyclidium* sp.

A different embodiment of the present invention concerns a procedure for the biotechnical phytodepuration of polluted waste comprising:

(a) providing a tank;

(b) providing inside the tank a filtering bed constituted of porous material and vegetable species planted on said filtering bed,

(c) passing the polluted waste through the tank, bringing it into contact with the filtering bed to obtain depurated waste;

in which the procedure envisions the inoculation of the filtering bed with a microorganism consortium comprising:

i) at least one symbiont fungus of the genus Glomus spp. or Gigaspora spp., and

ii) at least one saprophytic fungus of the genus Trichoderma spp., and

iii) at least one bacterium of the rhizosphere selected among the genera *Bacillus* spp., *Pseudomonas* spp. and *Actinomycetales* spp.

DETAILED DESCRIPTION OF SOME EMBODIMENTS

The invention will now be described, by way of example only, with reference to the single attached drawing, in which an embodiment of a phytodepuration system according to the present description is illustrated.

In the description that follows, numerous specific details are presented to provide a thorough understanding of the embodiment. The embodiments can be practiced without one or more of the specific details, or with other methods, components, materials, etc. In other instances, well-known structures, materials, or operation are not shown or described in detail to avoid obscuring aspects of the embodiments.

Reference throughout this specification to "one embodiment" or "an embodiment" means that a particular feature, structure or characteristic described in connection with the embodiment is included in at least one embodiment. Thus, the appearances of the phrases "in one embodiment" or "in an embodiment" in various places throughout this specification are not necessarily all referring to the same embodiment. Furthermore, the particular features, structures or characteristics may be combined in any suitable manner in one or more embodiments.

The headings provided herein are for convenience only and do not interpret the scope or meaning of the embodiments.

The physico-chemical methods most commonly used for treating wastes can be expensive and may not resolve the problem of their depuration.

The present description provides, for the first time, evidence for the advantages in terms of efficacy of the use of integrated systems constituted of microorganisms and detoxifying plants to overcome the limitations inherent in the single approach to phyto purification.

One embodiment of a phytodepuration system according to the present invention is schematically illustrated in the attached figure.

The phytodepuration system, indicated in its entirety with reference 1, comprises a tank 2, having an inlet and an outlet, the inlet being preferably at a height greater with respect to the outlet. Preferably the tank 2 is arranged beneath the level of the soil 3, rendered impermeable on its internal surfaces 4 with a sheet 5 of plastic material (such as, for example EPDM).

The tank 2 contains a filtering bed (or filling substrate) 6 constituted of highly porous material, where in a preferred embodiment such highly porous material consists of a mixture of clay, pyroclastic rock such as pumice, zeolites, limestone rock, diatomaceous earth, and/or sepiolites, where such filtering bed can also assume a structure with vertical and horizontal layers of diverse compositions.

Hydrophilic vegetable species 7 are planted on the filtering bed 6.

In addition, a consortium of fungi and/or bacteria useful for treating waste is periodically inoculated on the filtering bed 6.

Such system operates by submerged horizontal flow (SFS-h), where the waste to be treated is input through an input tube 10, preferably but not necessarily at a proximal extremity 11 of the tank 2, in correspondence with the above-said inlet; the depurated waste is then removed through the above-said outlet, preferably but not necessarily at a distal extremity 12 of the tank 2, by a draining tube 13 after flowing through the filtering bed 6 following a horizontal path favoured by a slight inclination of the bottom of the tank 2. The waste level inside the tank 2 is preferably maintained below the upper surface 15 of the filtering bed 6, at the same time guaranteeing saturation of the filtering bed with the waste. The phytodepuration system is also provided with means for managing the waste flow, which can for example comprise inspection wells 18 and 19. In the example illustrated, the upstream inspection well 18 serves to direct the wastes, also from different origins, downstream or into the tank 2. It is also possible to envision the existence of waste pre-treatment systems upstream of the phytodepuration system (Imhoff, septic tanks, sedimentation and separation systems for solids). The downstream inspection well 19 serves for sampling the output treated waste, for draining the tank 2 if needed and for regulating and maintaining the waste level in the tank 2.

The ratio between bed depth, width and length ensures the maximal hydraulic gradient available, necessary to provide adequate movement of the waste from the inlet towards the outlet without return or stagnation of the flow. The tank is gravity fed in a continuous or discontinuous way and normally lifting/pumping systems are not necessary.

A brief description of the various constituent elements of the phytodepuration system object of the present description is provided below.

The internal surface of the tank(s) 2 is impermeabilized using sheets 5 of plastic material such as for example a single piece of EPDM. The choice of a plastic material is based on the following factors:

- Ease of positioning also in the absence of specialised labour. Plastic membranes are available in large dimensions (up to 15 m in width and 60 m in length), with a consequent reduction in joints needed to be made at the worksite and shorter installation time.
- Plastic membranes are extremely flexible even at low temperatures and can stretch by more than 300%, permitting excellent adaptation to possible deformation of the placement site and to any degree of temperature change.
- The plastic membrane is an inert material with minimal environmental impact both in the production and application phases.

The filtering bed 6 has a mean height of 0.45 to 0.85 cm (wetted part from 0.40 to 0.75 cm).

The filtering bed 6 is preferably constituted of a mixture of clay, pyroclastic rock (pumice, zeolites, lapilli, etc.), limestone rock, diatomaceous earth and/or sepolite of high porosity (>50%) with a grain-size in the range comprised between 1 mm and 50 mm, preferably between 2 and 30 mm; said mixture comprising preferably clay, pumice, zeolytes, limestone rock and diatomaceous earth.

The filtering bed 6 can envision a structure of vertical and/or horizontal layers. In one embodiment the filtering bed 6 comprises at least two vertical layers and at least on horizontal layer. In the preferred embodiment illustrated, the phytodepuration system described herein comprises a plurality of vertical or flanking layers 21, 22, 23, 24 and 25. Such vertical layers comprising predominantly zeolites and volcanic rock and are arranged longitudinally in succession in decreasing order of grain-size (50-0.2 mm) (layers 21, 22, 23 and 24) substantially from the inlet towards the outlet for approximately two thirds of the length of the tank 2 and then with increasing grain-size (0.2-50 mm) to the outlet (layer 25); the vertical layers 21 to 25 are then preferably covered with a horizontal layer 26 with a height of approximately 15 cm (grain-size 2-4 mm)constituted predominantly of zeolites for absorbing odours and for planting the vegetable species. The superficial layer 26, together with layers 21 to 25, extend longitudinally for substantially the entire length of the tank 2.

The use of different types of constituent materials for the filtering bed (clay, pumice, lapilli, etc.) allow for the constitution of different biological niches, to which different microorganisms in the consortium inoculated in the filtering bed 6 can adapt. This allows the variability of the microbial flora of the niches to be increased, as a function of the different types of substrate used.

The hydrophilic vegetable species 7 planted on the filtering bed 6 are preferably chosen from evergreen and/or vegetable species with a vegetative rest cycle.

Among the evergreen vegetable species, *Cladium mariscus* and/or *Scirpus lacustris*, which are active over the entire course of the year, are preferred. Among the vegetable species with a vegetative rest cycle (also known as seasonal) *Arundo donax* (which takes up large quantities of water from the soil to support rapid growth that can reach 5 cm per day in the Springtime), Phragmites australis, Typha latifolia, Typha minima, Typha angustifolia, Juncus spp. and/or Schoenoplectus lacustris are preferred.

The vegetable species are periodically cut, restoring the depurative overall capacity of the system and as a consequence, its efficiency.

In a preferred embodiment, the consortium of fungi and/or bacteria useful for treating wastes inoculated on the filtering bed 6 comprises:

a) at least one symbiont fungus belonging to the genus Glomus spp. or Gigaspora spp.,

b) at least one saprophytic fungus belonging to the genus Trichoderma spp., and

c) at least one bacterium of the rhizosphere selected among the genera *Bacillus* spp., *Pseudomonas* spp. and *Actinomycetales* spp.

The consortium of fungi and/or bacteria can also comprise:

- at least one biosurfactant fungus (or yeast) selected among the genera *Acinetobacter* spp., *Pichia* spp., *Torulopsis spp., Candida spp., Saccharomyces spp., Schizonella spp., Ustilago spp. and/or*

- at least one biosurfactant bacterium selected among the genera *Agrobacterium* spp., *Serratia* spp., *Flavobacterium* spp., *Mycobacterium spp., Nocardia spp., Corynebacterium spp., Rhodococcus spp., Arthrobacter spp., Thiobacillus spp., Gluconobacter spp., Aspergillus spp., Alcanivorax spp.*

The consortium of fungi and/or bacteria can also comprise a natural consortium of protozoa inoculated with 800 g/m^2 of mud containing protozoa cultivated according to common techniques known in the art.

A natural consortium of protozoa advantageously useful in the context of the present phytodepuration system illustrated herein comprises protozoa, for example, of the genus *Vorticella spp*, *Opercularia spp.*, *Colpidium sp.*, *Satrophilus sp.* and/or *Cyclidium sp.*

The fungi *Trichoderma spp.* and *Glomus spp.* are particularly useful in that they are capable of degrading branched-chain carbon compounds.

The biosurfactant fungi and/or bacteria, such as for example *Pichia pastoris*, *Acinetobacter calcoaceticus*, *Acinetobacter radioresistens*, *Pseudomonas aeruginosa*, *Rhodococcus spp.*, *Bacillus subtilis, Candida bombicola, Alcanivorax borkumensis, Alcanivorax venetianus*, are able to render water-soluble the hydrophobic substances contained in the waste to be treated. The presence of such biosurfactant fungi and/or bacteria increases the metabolism of components that cause the high COD and/or BOD_5 of the treated waste, and consequently provide an efficient reduction of those parameters.

The presence of the above-listed yeasts has the dual role of limiting possible odours cased by the aerobic and anaerobic fermentations that take place within the phytodepuration system.

The use of a microorganism consortium according to the present description foresees that the consortium of fungi and/or bacteria are mixed with the top 30 cm of material constituting the filtering bed. The inoculum is composed of 20-30% symbiont fungi and rhizospheric bacteria at 15% of the total. The fungi and rhizospheric bacteria are usually present at a concentration of $1x10^7$ Colony Forming Units (CFU).

The diverse microorganisms (fungi and/or bacteria) inoculated in the filtering bed 6 can constitute different biological niches as a function of the type of material constituting the filtering bed (clay, pumice, zeolites, lapilla, etc.). This increases the variability of the microbial flora in the niches themselves, as a function of the different substrates used. In fact, each microorganism has a preference: for example the *Pseudomonas* favour pyroclastic type rock, while the actinomycetes favour clayey rocks.

It is particularly useful to repeat the inoculation with such consortium and preferably with microorganisms that produce biosurfactant substances, where such repetition is envisioned by means of adding the consortium directly to the waste to be treated every 10 days.

In addition to inoculating the filtering bed 6 with the above-said microbiological consortium, the Applicant has verified that it can be advantageous to inoculate also the vegetable species 7 of the phytodepuration system with a microbiological consortium of the rhizosphere.

The microbiological consortium of the rizosphere is essentially constituted of mycorrhizal fungi, bacteria, actinomycetes, saprophytic fungi and micromycetes. In particular, the plant roots establish symbiotic relationships with mycorrhizal fungi that assist the roots in absorbing nutritional substances and water from the soil and receive necessary sugars from the plant; when in symbiosis with the plant, they amplify the explorative capacity of the roots by approximately 600-800 times, multiplying the normal extension of the root apparatus. The roots also establish a cooperative relationship with bacteria, actinomycetes and saprophytic fungi that metabolise nutritive substances for the plant and live off of root exudates. It has been calculated that approximately 20% of the substances produced by the plant through chlorophyll-based photosynthesis are transported to the roots and used to feed its microbiological consortium.

The vegetable species of the active phytodepuration system described herein can be advantageously inoculated with a microbiological consortium of the rizosphere placed near the roots of the plant at the time of planting on the filtering bed, or at the time of planting, the addition of 30-50 cc/plant of a microbiological consortium of the rizosphere placed near the roots of the plant can be envisioned.

The functioning principle of the phytodepuration system object of the present description envisions the use of a plant capable of metabolising the polluting substances, together with a consortium of fungi and/or bacteria as identified above, which colonises the roots. This combination creates a plant-microbiological consortium system that is very efficient at absorbing and degrading many polluting substances, and optimises the efficiency of the system also for water insoluble compounds. This association allows the *in situ* degradation of numerous pollutants that are resistant to traditional phytodepuration. The result is a general depuration of wastes in the tanks where the appropriately selected plant-microbe system is applied.

Two microbiological consortia are particularly attractive for the efficacy of the phytodepuration system described herein.

A first microbiological consortium (referred to hereinafter as Consortium 1) to inoculate in the filtering bed of the phytodepuration system described herein comprises:

- 30% "crude inoculum", where the expression "crude inoculum" indicates a mixture of shredded roots, spores, mycelium and substrate from a cultivation tank containing spores and mycelium of symbiotic fungi preferably of the genus Glomus (for example G. mosseae, G. viscosum, G. intraradices), capable of stimulating the generation of colony forming points (endomycorrhizal arbuscules) on the roots of the vegetable species 7 at least at 30%.

- saprophytic fungi of the genus *Trichoderma* (for example *T. harzianum*);

- rhizospheric bacteria of the genus *Pseudomonas* spp., *Bacillus* spp. and/or *Actinomycetes* spp.;

- at least one biosurfactant fungus (or yeast) from the genera *Acinetobacter* spp., *Pichia* spp., *Torulopsis spp., Candida spp., Saccharomyces spp., Schizonella spp., Ustilago spp.;*

- at least one biosurfactant bacterium from the genera *Agrobacterium* spp., *Serratia* spp., *Flavobacterium* spp., *Mycobacterium spp., Nocardia spp., Corynebacterium spp., Rhodococcus spp., Arthrobacter spp., Thiobacillus spp., Gluconobacter spp., Aspergillus spp., Alcanivorax spp.*

- a natural consortium of protozoa cultivated according to common techniques known in the art.

This first consortium is in the form of powdery granules with a grain-size of 1-3 mm, and must be mixed with the material constituting the filtering bed or inoculated near the roots of the vegetable species planted on the filtering bed.

This consortium is inoculated, as is, into the filtering bed, preferably at a dosage of 7-20 $kg/1000m^2$, more preferably at 10-15 $kg/1000m^2$.

A second microbiological consortium (referred hereinafter as Consortium 2) to inoculate in the filtering bed of the phytodepuration system and to be used for the periodic reinforcing to maintain the necessary level of rizospheric microorganisms described here comprises:

- 10% crude inoculum (as defined above) capable of stimulating the generation of colony forming points (endomycorrhizal arbuscules) on the roots of the vegetable species 7 at least at 30%.
- saprophytic fungi of the genus *Trichoderma* (for example *T. harzianum*);
- rhizospheric bacteria of the genus *Pseudomonas* spp., *Bacillus* spp. and/or *Actinomycetes* spp.;
- at least one biosurfactant fungus (or yeast) from the genera *Acinetobacter* spp., *Pichia* spp., *Torulopsis spp., Candida spp., Saccharomyces spp., Schizonella spp., Ustilago spp.;*
- at least one biosurfactant bacterium from the genera *Agrobacterium* spp., *Serratia* spp., *Flavobacterium* spp., *Mycobacterium spp., Nocardia spp., Corynebacterium spp., Rhodococcus spp., Arthrobacter spp., Thiobacillus spp., Gluconobacter spp., Aspergillus spp., Alcanivorax spp.*

This second consortium is in the form of a wettable powder (WP) with a grain-size of about 100 µm. It is diluted in an appropriate amount of water and used once every 10 days at a dosage comprised between 200 and 400 g/1000 m^2 , preferably between 200 and 300 g/1000 m^2, more preferably between 200 and 220 g/1000 m^2.

The presence of the above-listed yeasts in the two consortia has the dual role of limiting possible odours caused by the aerobic and anaerobic fermentations.

Naturally, while the principle of invention remains constant, the structural details and the embodiments may vary, even appreciably, with reference to what has been described and illustrated by way of example only, without departing from the scope of the present invention.

CLAIMS

1. A biotechnological phytodepuration system (1) for polluted waste, the system comprising a tank (2) containing a filtering bed (6) constituted of porous material and vegetable species (7) planted out onto said filtering bed (6), an inlet (10) for introduction of polluted waste into said tank (2) and an outlet (13) for discharge of depurated waste from the tank (2), the system being characterized in that said filtering bed (6) is inoculated with a microorganism consortium, said consortium comprising:

a) at least one symbiont fungus belonging to the genus Glomus spp. or Gigaspora spp.,

b) at least one saprophytic fungus belonging to the genus Trichoderma spp., and

c) at least one bacterium of the rhizosphere selected among the genera *Bacillus* spp., *Pseudomonas* spp. and *Actinomycetales* spp.

2. The system according to claim 1, wherein said microorganism consortium further comprises:

- at least one biosurfactant fungus (or yeast) selected among the genera *Acinetobacter* spp., *Pichia spp., Torulopsis spp., Candida spp., Saccharomyces spp., Schizonella spp., Ustilago spp. and/or*

- at least one biosurfactant bacterium selected among the genera *Agrobacterium* spp., *Serratia* spp., *Flavobacterium* spp., *Mycobacterium spp., Nocardia spp., Corynebacterium spp., Rhodococcus spp., Arthrobacter spp., Thiobacillus spp., Gluconobacter spp., Aspergillus spp., Alcanivorax spp.*

3. The system according to claim 1 or claim 2, wherein said microorganism consortium further comprises a natural consortium of protozoa.

4. The system according to any one of the preceding claims, wherein said filtering bed (6) comprises at least one among: clay, pyroclastic rock, limestone rock, diatomaceous earth, sepiolite and/or mixtures thereof.

5. The system according to any one of the preceding claims, wherein said vegetable species (7) are selected among:

- evergreen species selected among *Cladium mariscus*, and *Scirpus lacustris*, and/or
- species with a vegetative rest cycle selected among *Arundo donax, Pragmithes australis, Typha latifolia*, *Typha minima*, *Typha angustifolia*, *Juncus* spp., and *Schoenoplectus lacustris*.

6. The system according to any one of the preceding claims, wherein said filtering bed (6) is constituted of porous material having a porosity greater than 50% and grain-size comprised between 1 and 50 mm, preferably between 1 and 30 mm.

7. The system according to any one of the preceding claims, wherein said filtering bed (6) has a structure comprising horizontal (26) and/or vertical (21, 22, 23, 24, 25) layers.

8. The system according to any one of the preceding claims, wherein crushed roots, spores and mycelia of mycorrhizal fungi are added to said microorganism consortium.

9. The system according to any one of the preceding claims, wherein said microorganism consortium is in form of powdery granules or wettable powder.

10. The system according to claim 9, wherein said microorganism consortium in the form of powdery granules is inoculated into said filtering bed (6) in an amount comprised between 7 and 20 Kg/1000 m^2, preferably between 10 and 15 Kg/1000 m^2.

11. The system according to claim 9, wherein said microorganism consortium in the form of wettable powder is inoculated into said filtering bed (6) in an amount comprised between 200 and 400 g/1000 m^2, preferably between 200 and 300 g/1000 m^2.

12. The system according to any one of the preceding claims, wherein said microorganism consortium is periodically inoculated into said filtering bed (6), preferably every 20 days, more preferably every 10 days.

13. The system according to any one of the preceding claims, wherein said vegetable species are inoculated with a microbiological consortium of the rhizosphere before or at the moment of planting them out onto the filtering bed (6).

14. A method for the biotechnological phytodepuration of polluted waste, comprising:

(a) providing a tank (2);

(b) providing a filtering bed (6) inside the tank (2) constituted of porous material and vegetable species (7) planted out on said filtering bed (6),

(c) passing the polluted waste through the tank (2), bringing it into contact with the filtering bed

(6) to obtain depurated waste;

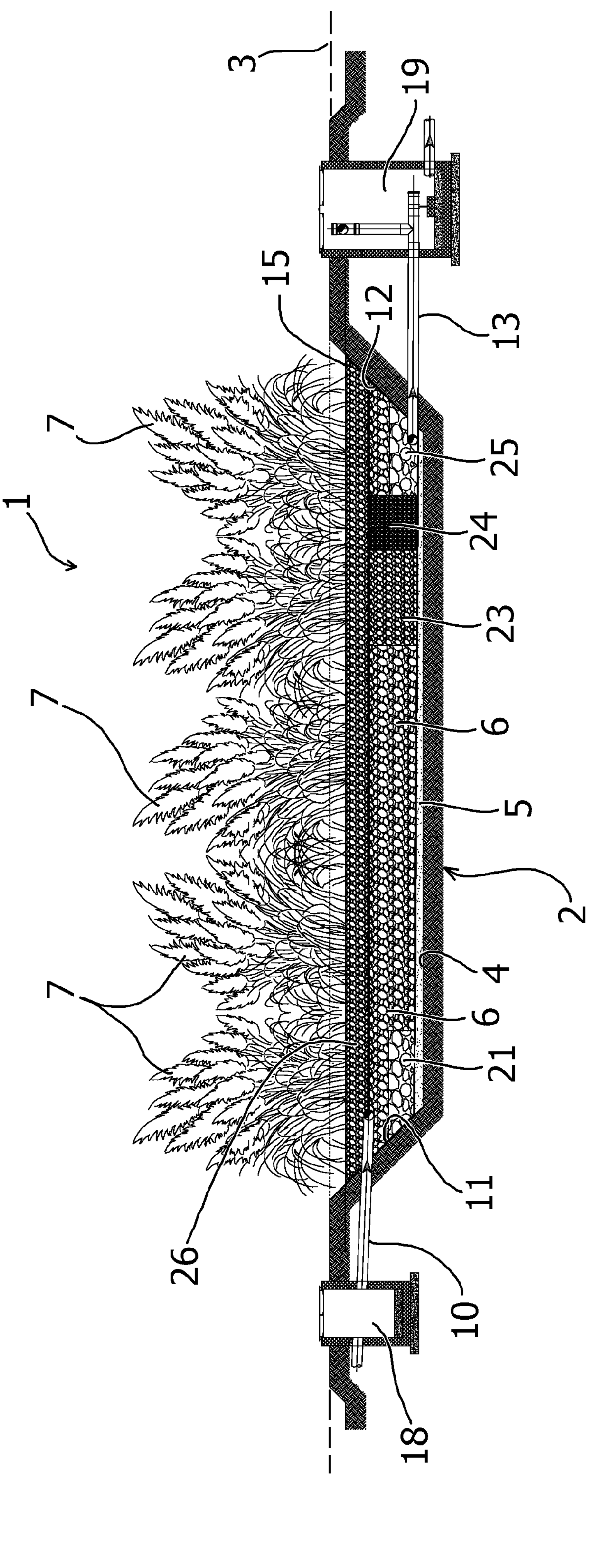
the method comprising the inoculation of said filtering bed (6) with a consortium of microorganisms comprising:

i) at least one symbiont fungus of the genus Glomus spp. or Gigaspora spp., and

ii) at least one saprophytic fungus of the genus Trichoderma spp., and

iii) at least one bacterium of the rhizosphere selected among the genera *Bacillus* spp., *Pseudomonas* spp. and *Actinomycetales* spp.

15. The method according to claim 14, in which the polluted waste is made to pass between an inlet and an outlet of the tank (2) by means of submerged horizontal flow or (SFS-h).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INTERNATIONAL SEARCH REPORT

International application No
PCT/IB2011/051083

A. CLASSIFICATION OF SUBJECT MATTER
INV. C02F3/32 C02F3/34
ADD.

According to International Patent Classification (IPC) or to both national classification and IPC

B. FIELDS SEARCHED

Minimum documentation searched (classification system followed by classification symbols)
C02F B09C C09C

Documentation searched other than minimum documentation to the extent that such documents are included in the fields searched

Electronic data base consulted during the international search (name of data base and, where practical, search terms used)

EPO-Internal, WPI Data

C. DOCUMENTS CONSIDERED TO BE RELEVANT

Category*	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
X	US 5 951 866 A (GROVE JOHN E [US] ET AL) 14 September 1999 (1999-09-14) cited in the application column 5, line 47 - line 65; figure 1	1-15
X	US 2007/261299 A1 (KEPHART PAUL [US]) 15 November 2007 (2007-11-15) paragraph [0061] - paragraph [0082]	1-15
X	WO 2008/029423 A1 (CCS AOSTA S R L [IT]; GIOVANNETTI GIUSTO [IT]) 13 March 2008 (2008-03-13) page 3, line 10 - line 14 page 15, line 12 - page 16, line 33 table 1	1-15

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[X] Further documents are listed in the continuation of Box C.

[X] See patent family annex.

* Special categories of cited documents :

"A" document defining the general state of the art which is not considered to be of particular relevance

"E" earlier document but published on or after the international filing date

"L" document which may throw doubts on priority claim(s) or which is cited to establish the publication date of another citation or other special reason (as specified)

"O" document referring to an oral disclosure, use, exhibition or other means

"P" document published prior to the international filing date but later than the priority date claimed

"T" later document published after the international filing date or priority date and not in conflict with the application but cited to understand the principle or theory underlying the invention

"X" document of particular relevance; the claimed invention cannot be considered novel or cannot be considered to involve an inventive step when the document is taken alone

"Y" document of particular relevance; the claimed invention cannot be considered to involve an inventive step when the document is combined with one or more other such documents, such combination being obvious to a person skilled in the art.

"&" document member of the same patent family

Date of the actual completion of the international search	Date of mailing of the international search report
29 July 2011	04/08/2011

Name and mailing address of the ISA/	Authorized officer
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INTERNATIONAL SEARCH REPORT

International application No
PCT/IB2011/051083

C(Continuation). DOCUMENTS CONSIDERED TO BE RELEVANT

Category*	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
A	JP 5 168348 A (CENTRAL GLASS CO LTD) 2 July 1993 (1993-07-02) abstract	1-15
A	US 2002/121046 A1 (YAMASHITA THOMAS T [US]) 5 September 2002 (2002-09-05) example 8	1-15
A	AVIS T J ET AL: "Multifaceted beneficial effects of rhizosphere microorganisms on plant health and productivity", SOIL BIOLOGY AND BIOCHEMISTRY, PERGAMON, OXFORD, GB LNKD- DOI:10.1016/J.SOILBIO.2008.02.013, vol. 40, no. 7, 1 July 2008 (2008-07-01), pages 1733-1740, XP022733623, ISSN: 0038-0717 [retrieved on 2008-05-06] abstract	1-15
A	EP 1 414 756 A1 (MARCHELLO GIOVANNI BATTISTA [IT]) 6 May 2004 (2004-05-06) cited in the application the whole document	1-15

INTERNATIONAL SEARCH REPORT

Information on patent family members

International application No

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