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(71) Applicant (for all designated States except US):
R.A.C.E. S.R.L. [IT/IT]; Via Gaetano Donizetti 36,
I-20122 Milano (IT).

(72) Inventors; and

(75) Inventors/Applicants (for US only): **ROMANI, Annalisa** [IT/IT]; Via delle Lame, 3, I-51031 S.Michele Agliana (PT) (IT). **PANGIA, Domenico** [IT/IT]; R.A.C.E. s.r.l., Via Gaetano Donizetti 36, I-20122 Milano (IT). **MARCHIONNI, Luisa** [IT/IT]; R.A.C.E. s.r.l., Via Gaetano Donizetti 36, I-20122 Milano (IT). **MARCHIONNI, Alessandro** [IT/IT]; R.A.C.E. s.r.l., Via Gaetano Donizetti 36, I-20122 Milano (IT).

(74) Agents: **BANCHETTI, Marina** et al.; Barzano' & Zanar-
do Roma S.p.A., Via Piemonte, 26, I-00187 Roma (IT).

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(57) Abstract: A process for the integral recovery of components and chemical compounds from olive mill wastewaters and olive husks with membrane technologies, resins and treatments of the dephenolised material product with anaerobic and aerobic attached-biomass columns and with aerobic tray separation column, as a preliminary phase for exploiting said wastes for the production of biogas and fertilizers having high-quality and high availability in the soil. The disclosed treatment is characterized by the following steps: pre-dilution, centrifugation; selective fractionation for the recovery of polyphenol compounds through a membrane process and adsorption on resins; pretreatment in aerobic and anaerobic attached-biomass columns and tray separation column; anaerobic biodigestion for biogas and fertilisers production; cogeneration for the production of a thermal and electrical power vector; production of water reusable in the process, having hydro-chemical characteristics suitable to civil use.



INTEGRATED PROCESS FOR RECOVERY OF A POLYPHENOL FRACTION AND ANAEROBIC DIGESTION OF OLIVE MILL WASTES

SPECIFICATION

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The present invention concerns an integrated process for recovery of a polyphenol fraction and anaerobic digestion of olive mill wastes. More specifically, the invention concerns a treatment for the integral recovery of components and chemical compounds present in the olive mill waste waters and in the olive husks by means of membrane technologies and resins, followed by treatments of the dephenolised olive mill wastes on aerobic and anaerobic columns, as a preliminary step for the treatment thereof for biogas and high quality fertilizers having a good bioavailability in the soil. Specifically, the invention provides a process having the following steps: pre-dilution, centrifuga-
10 tion; selective fractionation for the recovery of polyphenol compounds through a membrane process and adsorption on resins; pretreatment in aerobic and anaerobic attached-biomass columns and tray separation column; anaerobic biodigestion for biogas and fertilisers production; cogeneration for the production of a thermal and electrical power vector; production of water reusable in
15 the process, having hydrochemical characteristics suitable to civil use.

As is known, olive oil production generates every year considerable amounts of wastewater, i.e., the olive mill wastewater (OMW), as well as great amounts of solid waste, i.e. the olive husks residues. The disposal of such wastes represents a burden resting with the enterprises and one of the biggest environmental problems of the Mediterranean region. Although they are of a natu-
25 ral origin, the wastes produced by the olive-oil industry have a great polluting impact. The OMW appear as a dark, strongly acid (pH 5-5.5) and unpleasant-smelling liquid, that is easily fermentable, and essentially consists of the juice present in the olive fruits, of the dilution and washing waters and of a modest
30 oil residue, amounting to 0.5% on average. In detail, the OMW contain mineral salts - mainly potassium and phosphate salts - and organic substances such as fats, proteins, sugars, pectins, gums and polyphenols. They have a high

microbial load and consist, on average, of 83.4% water, 1.8% mineral salts and 14.8% organic compounds.

The organic compounds, in particular polyphenols, are actually responsible for the polluting potential of OMW: as a matter of fact, these are substances subject to oxidation, which may alter the environmental conditions of a possible receiving body. They can cause acidification, saturation, stagnation, impermeabilisation, inhibition and intoxication; therefore, the olive mill wastewaters, if directly introduced in the water streams, would in very short times induce anoxia phenomena due to a depletion of oxygen dissolved in water, thus damaging not only the water fauna, but also the aerobic microorganisms, that need oxygen to perform their self-depurating action. Due to said aspects, any direct disposal of such wastes in the sewage system is not allowed, while only some limited amounts thereof can be employed for fertilizing irrigation (50 m³/ha for OMW coming from traditional discontinuous pressing oil mills and 80 m³/ha for OMW coming from the continuous cycle.)

To that regard, in spite of the fact that minimal amounts of polyphenols are metabolised by the microbial flora of soils, some negative effects have been ascertained, such as immobilisation of available nitrogen, amphisol formation due to the substitution of potassium in calcium complexes, salinity increase and reduction of magnesium available to the plants, very likely due to the antagonist effect of potassium.

In general, the quality and quantity of the various OMW components is different depending on the type of apparatus employed for oil extraction.

In the traditional extraction system by pressing in batch a limited amount of water is obtained, while the centrifugation (or continuous) system needs a greater amount of water in order in order to fluidify the paste in the extraction step and to assist the oil expulsion. The need to improve the oil quality while reducing the environmental impact of the OMW generated further study on the possibility of modifying the "three-phase" centrifugal extraction system so as to reduce the addition of process water.

For the above reason the so called "two-phase" extraction systems have been introduced, where no water is added in the decanter and the by-

product obtained consists of one only phase, made up by a mixture of husks and waste-waters. This improvement, however, has no effect on the reduction of the environmental impact and does not allow a total solution of the problem, so that further research and experimental applications have been boosted
5 towards innovative solutions for the purification and disposal of such residues, such as, for instance, membrane separation processes (e.g., ultrafiltration and reverse osmosis).

In the membrane separation processes, however, the high concentration of suspended solids and colloidal materials of the OMW makes the passage through the membrane quite difficult, resulting in clogging problems. It is
10 also possible to exploit the olive oil industry wastes in anaerobic biodigestion processes. Such fermentation processes afforded interesting results both in terms of COD abatement (91%), and as regards methane production (0.80 g CH₄/g total of COD), but present some problems due to the slow biochemistry
15 of the anaerobic process. Actually, the microbial transformation processes are inhibited by the presence of polyphenols; in particular, the acetogenic and methanogenic bacteria are sensitive to concentrations >0.25 g/l of caffeic acid and >0.12 g/l of *p*-coumaric acid.

It is to be observed, however, that if on one hand such molecules are
20 environmentally phytotoxic and inhibit the fermentation processes, on the other hand they have a high antioxidant activity, and are active as anti-atherogenic, coronary-dilating, anti-cholesterolemic and hypoglycemic agents, suitable for use in the food industry as well as in the cosmetic and phytotherapeutic field. Polyphenol compounds such as tyrosol and hydroxytyrosol, oleuropein, aglycon and the relevant hydrolysis and/or rearrangement products,
25 phenolic acids, apigenin and luteolin (Romani et al., 2002), being transferred only in minor amounts in the oil during the oil production process (150-400 mg/l), are water-soluble, and thus they remain on large amounts in the olive mill wastes. The foregoing suggests a possible way for an interesting economic
30 recovery, deriving from valorisation and marketing of such molecules, which may be isolated from the concerned waste material. The foregoing allows regarding the OMW as a "*secondary starting material*" for innovative

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productions rather than a *waste product*.

At present, one of the technologies having the widest valorisation range for the concerned wastes is the Aquatec technology (Aquatec 3 W GmbH, Dresden, DE - A3w and AquatecOLIVIA technologies). The latter
5 affords the transformation of solid and fluid wastes of the olive oil production into energy and resources. The modular plant proposed in connection with said technology is based on three process lines: water purification; sludge treatment and biogas exploitation.

The plant may treat the following elements:

- 10 ▪ olive mill wastewaters;
- olive husks from 3-phase and 2-phase systems;
- low value olive oils (for instance, deriving from the chemical working of husks, other vegetable oils, or wastes of organic fats);
- wastewaters and organic residues from dairies, slaughterhouses and
15 farms.

Such substances are transformed and valorised into the following products: olive oil (food quality); biodiesel (fuel); olive stones (combustible); pellets (combustible, fertilizer, animal feed); biogas (electrical power, heat, cold, and steam); irrigation water; compost.

20 According to the Aquatec technology, the main production lines are the following ones:

- **wastewater treatment** (fluid phase): physical/biological process and methanogen fermentation of the organic substances in the waste water with biogas production;
- 25 ▪ **biogas valorisation** (gaseous phase): the biogas resulting from the treatment of the waste waters is transformed into thermal energy and covers the internal heat requirements;
- **olive husks recovery** (solid phase): such process comprises the extraction of olive oil by centrifugation, the extraction of the olive stone by
30 centrifugation, and the drying of the flash (in a drum or a fluidised bed dryer) with subsequent transformation into pellets. A further possibility for valorisation of the husks may derive from compost production proc-

esses and fermentation for biogas production.

Figure 1 of the attached drawings shows a three-dimensional schematic representation of a platform according to the cited prior art. If, on one hand, the bio-refinery described above combines the treatment of the OMW with the recovery of matter and power, on the other hand it presents some inefficiencies in terms of productive continuity and energetic yield.

In the light of the foregoing, an object of the present invention is to provide an integrated platform, efficient and multifunctional, which allows to carry out in a preliminary phase the extraction and recovery of bioactive components from the olive mill wastes and, at the same time, to eliminate in an effective manner the polluting load of the residual materials, with production of energy in the form of biogas and high-quality fertilizers.

According to the invention, there is proposed an integrated plant complex (or platform) which comprises in an innovative way the following phases: pre-dilution, centrifugation, selective fractionation for the recovery of polyphenol compounds by means of a membrane process and adsorption on resins; pretreatment with aerobic and anaerobic attached-biomass columns and tray separation column; anaerobic biodigestion for biogas and fertilizer production; cogeneration for the production of a thermal and electrical power vector; production of water reusable in the process, having hydro-chemical characteristics suitable to civil use.

Such platform comprises a phase of anaerobic biodigestion of the OMW for the production of biogas, while including however a waste water pretreatment step, which allows the standardisation of the incoming biomass and of the inoculum necessary to start the fermentation. The pretreatment process allows, in addition, to obtain an optimised energetic yield, the productive continuity and high-quality fertilizers, thus resulting in a technological innovation with respect to already existing platforms.

The production of polyphenol extracts from the OMW is based on a refining process taking advantage from the use of membrane separation technologies and resins. The extraction technology allows to obtain the following products: ultrapure water, to be recycled in the productive process or to be

used for the market of functional beverages; a raw fraction deriving from the microfiltration retentate, valorisable for the biogas production together with preliminary phase by-products; more different types of extracts, differing both in the title and in the presence of molecules of different activity and biological effectiveness, and therefore endowed with different possibilities of exploitation.

The molecules present in the fraction with higher biological interest are hydroxytyrosol and its derivatives, as well as verbascoside and its derivatives. Hydroxytyrosol is a powerful antioxidant and cardio-protective agent (Covas M.I., et al., *Nutr. Rev.* 2006; 64:S20; Visioli et al., *Biochem. Biophys. Res. Commun.*, 1998; 247:60), and is the compound having the highest functional activity in extra-virgin olive oil. It delays the LDL oxidation (Visioli F., Galli C., *Life Sci.* 1994; 55:1965) and it has also been reported to be able to reduce the gene expression of iNOS and COX-2 in cell lines, preventing the activation of the transcription factors NF-KB, STAT-1 α and IRF-1 (Maiuri et al., *Naunyn-Schmiedeberg's Arch. Pharmacol.* 2005; 371:457). According to some authors it also inhibits the platelets aggregation *in vitro* (Petroni et al., *Thromb. Res.*, 1995; 78:151).

The literature provides several studies concerning the biological activities of molecules having phenylpropanoid structure, in connection with their antioxidant, anti-inflammatory, antiviral and anti-mycotic activity. Most of such studies are mainly focused to evaluate the effects of verbascoside or acteoside, that is one of presently most studied phenylpropanoids. Verbascode has also evidenced an *in vitro* immunomodulatory activity, in particular it increases the chemotactic activity of neutrophils; an opposed effect has been observed on increasing the dose, mainly due to a suppression of the killer activity of the neutrophil lymphocytes (Akbay P. et al., *Phytother. Res.* 2002; 16:593). Further, an anti-neoplastic activity, both for verbascoside and for its isomer isoacteoside, are also reported. From *in vivo* test, carried out on leukemic murine cells P388, both phenylpropanoids have shown a cytotoxic activity at ED₅₀ concentration of 10 μ g/mL for isoverbascode and 26 μ g/mL for verbascoside (Pettit G.R. et al., *J. Nat. Prod.* 1990; 53:4561990).

From the results obtained in laboratory and from preliminary market analyses there appears the possibility and at the same time the interest of proposing *Olea europaea* extracts that are able to differentiate with respect to competitor products both in terms of molecules present. (e.g. verbascoside) and in terms of their concentration. In particular, from the proposed type of extraction it is possible to obtain, starting from OMW and when present, from olive husks, several fractions, with a different content of active principles coming from the flash of de-stoned olives.

The innovative elements characterizing the present invention with respect to the technology nowadays existing technologies are essentially the following:

1. it is a technology able to treat olive oil production wastes coming both from two-phase and from three-phase olive mill;
2. the sequence of integrated technologies, as described further on, allows to standardize the biomass entering the anaerobic biodigester, thank to the pretreatment and to the control, with analytic techniques, of total polyphenols and ortho-diphenols, which are toxic molecules that inhibit anaerobic process;
3. a further innovation derives from the standardization of the inoculums, which allows the process continuity without penalizing the biogas yields.

In addition, it is possible to foresee, as an innovative aspect, the mixture of olive industries wastes with whey, animal excrement and with about 10% of solid organic municipal waste.

The process refers to a standard composition of the OMW and olive husks (substrate), obtained separately or (as in the case the two-phases process) in a mixture. Such compositions may vary depending on:

- oil extraction technique
- olives variety
- period of collection
- type of treatment for the extraction of hydroxytyrosol, polyphenols, dyes, etc.

The composition variability, however, does not change the treatment technology and the particular features of the technological solutions; it only influences the mass and energy balance of the system:

- biogas produced per weight unity of treated waste;
- 5 ▪ energy spent per mass transfer;
- hydraulic and biomass retention time.

The process description is reported in the following.

DESCRIPTION OF THE FIGURES

The figures of the enclosed drawings represent some exemplificative
10 embodiments related to the invention, and more specifically:

Figure 1 represents an example of multifunctional platform for the production of biogas from waste materials of *Olea europea* L. according to the integrated process of the cited prior art:

Figure 2 shows a block diagram of the central section of the inte-
15 grated process of the OMW treatment according to the invention;

Figure 3 shows a simplified process scheme with the first section of the integrated plant according to the invention, comprising the central part of Figure 2 and a preliminary section for the separation of polyphenol compounds,

20 **Figure 4** shows a simplified process scheme with the second section of the integrated plant according to the invention, used for cogeneration and fertilizer production;

Figures 5 and 6 show the calibration curves for the analytic control techniques used in the process.

25 DETAILED DESCRIPTION OF THE INVENTION

Waste treatment before the anaerobic digestion

The pH of incoming olive mill wastewaters is adjusted to values in the range from 3 to 4.5, in order to inactivate the polyphenol oxidase enzyme, which would tend to oxidize the useful polyphenol compounds. The, the OMW
30 or the waste products from the two-phase olive oil extraction undergo a preliminary treatment as shown in **Figure 3**: pre-dilution, pre-oxidation and centrifugation, selective fractionation for the recovery of polyphenol compounds by

means of a membrane process followed by and adsorption on resins; pre-treatment in aerobic and anaerobic attached-biomass columns and in a tray separation column.

- The **pre-dilution and pre-oxidation step** is intended to oxidize part of the excess polyphenols in order to prevent them from causing process blockages in the subsequent fermentation and, by means of the addition of acidifying agents or proteolytic enzymes, is aimed at potentiating the release of polyphenol compounds from the solid particles, besides catalyzing the hydrolysis of secoiridoids and of some complex molecules;
- 10 - the **centrifugation step** is applied both to OMW from three-phase mill and in the case that OMW from two-phase mill are treated (in this case this step is preceded by a pre-dilution of the moist olive husks with process water, as previously described for the **pre-dilution and pre-oxidation step**);
- **tangential microfiltration (MF)** of the surnatant, followed by a **tangential ultrafiltration (UF)** treatment carried out on the microfiltration permeate; the retentate coming from the MF partly undergoes again the pre-dilution and partly, mixed with the UF retentate, is reprocessed in centrifuge in order to undergo further cycles of extraction of polyphenols from the moist husks; the UF permeate is in turn fed to the treatment with resins;
- 15 - **treatment with polymeric resins and water** : from the said treatment it is possible to obtain purified fractions enriched in polyphenols by means of an operation of adsorption-desorption on inert polymeric resin; desorption may be carried out either with ethanol or with acidic and non acidic water;
- **analysis before entering the pretreatment columns** (attached biomass columns, CAA and CAN, and tray column, CAS) in order to standardize the mass for the biogas production and thus obtain information on its yield. The analytic control techniques applied, which are described in the paragraph reported below ("Analytic control techniques"), are able to provide the following information:
- 25
- 30
 - Carbon title;
 - Nitrogen title;
 - Carbon/nitrogen ratio;

- Total polyphenols (10 % max) and ortho-diphenols (5% max);
- Presence of microelements.

The previous treatment phases, i.e.: **pre-dilution and pre-oxidation, centrifugation, microfiltration and ultrafiltration, treatment on polymeric resins and with water, analysis before entering the pretreatment columns** for the control of total polyphenols and ortho-diphenols, are subsequently repeated on the dilution and washing waters of the husks after first centrifugation of the waste and possible mixture with the MF retentate. As dilution and washing waters for the solid obtained from first centrifugation, the waters obtained from percolation from resins (**Figure 3**) are used, or those from reverse osmosis concentration, as described in the further phases of the process (**Figure 4**).

If the control of the solid after second centrifugation shows a content of total polyphenols lower than 10% and a content of ortho-diphenols lower than 5%, the solid residue (i.e., Fp, primary sludge, in Figures 2 and 3) is directly fed in the first anaerobic digester (DG1) in mixture with the liquid waste pretreated as described above.

Preliminary and supplementary treatments of the anaerobic digestion

- Anaerobic tray separation column (CAS)

On exiting the methanogen anaerobic digester (DG3) the substrate is fed to a tray contact and separation column: such column works in anaerobic conditions.

In this column, a concentration gradient of suspended solids decreasing from the bottom to the top is created. An elementary section of the column is represented by a zone comprised between two trays: the clarified fluid is transferred towards the top (i.e. at the following tray) by gravity. The single trays are slightly at a slope and present an aperture in the higher part: the various apertures are aligned on the same axis. The gap between one tray and the other represent the settling zone, with a hole for the discharge for the sludge discharge. The said holes are in communication with the main reactor DG3.

According to some specific embodiments of the invention the geome-

trical features of the trays are as follows:

- a) The holes have a variable diameter, not lower than 1 cm and not higher than 10 cm;
- b) The ratio of the fluid rates through the apertures for the up-flowing fluid (clarified fluid) and those through the holes for the sludge is not lower than 5.

The fluid with the highest concentration of suspended solids is returned to the methanogen reactor, while the clarified fluid passes to the following anaerobic attached biomass column.

10 - **Anaerobic attached biomass column (CAN)**

Such column is a multilayer packed column, with the following packing;

- perforated bricks
- sand
- 15 - activated carbon.

The function of the inert packing is to supply an anchoring to the biomass and thus create some zones (biofilm) wherein a very high concentration of biomass occurs; this results in minimising the inhibition factors. The activated carbon, in addition, performs a selective adsorption action, mainly in respect of enzyme complexes, while the bricks have a more complex function:

- adsorption;
- supply of microelements useful to inorganic biocatalysis;
- fluid dynamic effect.

25 The fluid dynamic effect is made evident by the separation of suspended solids and by their deposition on the brick walls, and it is due to acceleration and deceleration phenomenon that the fluid particles undergo while passing through the channel system of the brick.

Thus, the bricks packing has a complex function, that may be synthesised as follows:

- 30 - it causes the formation of a superficial layer which serves to accumulate and hold biomass and bacteria;
- the rates of bacterial catabolism and anabolism are increased in the

biomass layer between the bricks and the fluid, since an exchange of matter is established: the brick supplies inorganic microelements and adsorbs macromolecular components.

This not fully clarified phenomenon brings about an increase in the
5 reaction rates and a decrease of the biotoxicity factors.

The fluid exiting the column is returned in part to the methanogenic reactor, and in part is fed the following aerobic column (CAA).

- **Fluidized bed aerobic attached biomass column (CAA)**

The clarified fluid exiting the previous column (CAN) goes to a fluid-
10 ized bed combined aeration column fed with the dephenolised OMW and husks (AVSd). The fluidized bed is made out by sand and activated carbon. As it is known, the fluidized bed has the function of a biomass fixator, while the function of activated carbon is not fully clear. Very likely, the activated carbon:

- 15 - exerts an effective action of biomass accumulation; and
- exerts very important function of adsorption, which results in a remarkable increase of the retention time of certain macromolecules in the system; this increase in the retention time justifies the global result of increased degradation of certain components which would
20 otherwise be considered resistant.

The fluid exiting the CAA column reverts to the first stage of the plant.

Preferably, the microorganisms pool to be periodically inoculated in the reactors, and precisely in the CAA and CAN columns and in the DG2 digester, may consist of the following microorganisms:

- 25 1. methanogen bacteria: isolated from the CAN column medium;
adapted collection microorganisms:

Methanosaeta concihi

Klebisella oxytoca

Clostridium propionicum

Leuconostoc mesenteroides

Clostridium butyricum

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Desulfovibrio vulgaris

Methanobacterium formicicum

Methanohalobium barkeri

2. hydrolytic and hydrogen-productive bacteria: isolated from the DG2 anaerobic digester medium

3. aerobic microorganisms: isolated from the CAA column medium adapted collection microorganisms

Candida rugosa

Candida tropicalis

Phanerochaete chrysosporium

Pleurotus ostreatus

Laeptiporus sulphureus

Pleurotus sajor-caju

Funalia troglia

Paecilomyces variotii

Lentinus tigrinus

5 Anaerobic digestion phase and biogas production

The process starts from dephenolised OMW and husks (AVSd), and refers to a standard composition referred to average values as defined in the scientific literature, and to substrates actually employed in the tests.

The parameters considered are those significant per the anaerobic digestion process and for the production of fertilizers.

a. Composition (AVd)

OMW resulting from the extraction of antioxidants, dyes

	-TOC	mg/kg C	24000
	-COD	mg/kg O	60000
15	-BOD	mg/kg O	30000
	-total dry substances (SST)	%	5.1
	-suspended dry substances (SSS)	%	1.6
	-volatile substances (SSV)	%	3.9
	-total phosphates	mg/kg P	525
20	-total nitrogen	mg/kg N	375

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	-potassium	mg/kg K	5625
	-magnesium	mg/kg Mg	233
	-calcium	mg/kg Ca	165
	-sulphates	mg/kg S	445
5	-chlorides	mg/kg Cl	967
	-sodium	mg/kg Na	120
	-boron	mg/kg B	8
	-cobalt	mg/kg Co	0.04
	-iron	mg/kg Fe	48
10	-manganese		
	-molybdenum		
	-zinc	mg/kg Zn	48
	-selenium		
	b. Composition (Sd)		
15	Husks resulting from the extraction of antioxidants, dyes		
	-fatty substances	% ss	6.0
	-ashes	%	
	-COD	mg/kg O	
	-BOD	mg/kg O	
20	- total dry substances (SST)	%	20
	- volatile substances (SSV)	%	19
	-total phosphates	mg/kg P	1000
	-total nitrogen	mg/kg N	25000
	-potassium	mg/kg K	
25	-magnesium	mg/kg Mg	
	-calcium	mg/kg Ca	
	-sulphates	mg/kg S	
	-chlorides	mg/kg Cl	
	-sodium	mg/kg Na	
30	-boron	mg/kg B	
	-cobalt	mg/kg Co	
	-iron	mg/kg Fe	

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-manganese

-molybdenum

-zinc

mg/kg Zn

-selenium

- 5 Note : in the case of a two-phase extraction process the substrate will have the weight average composition of the two separate substrates; the variations concern the water content only.

c. Anaerobic digestion

- 10 The anaerobic digestion is a process carried out over liquid or semi-solid fractions (substrate), on which anaerobic microorganisms grow. Due to such microbial activity the organic substance is degraded and methane and other minor gases (carbon dioxide, hydrogen, hydrogen sulphide, etc.) are produced.

- 15 Said microbial reactions occur in reactors having variable dimensions, either single-stage reactors or multistage reactors, in absence of air and in a range of thermal and fluid-dynamic conditions defined as follows:

- temperature from 30 to 60°C.
- pH from 5.0 to 8.5
- stirring: dissipated energy from 0 – 0.6 kWh/m³ reactor/hr

- 20 The proposed process according to the invention is carried out, preferably, in two digestion stages: a preliminary anaerobic stage (DG1), a second stage (DG2) carried out in anaerobic conditions and with an inoculum of hydrolytic and hydrogen producing microorganisms, previously listed under item 2 (acidophilic stage) and a third stage (DG3) anaerobic as well, wherein
- 25 the biogas production mostly takes place (methanogenic stage).

- The second stage is characterised by biochemical reactions leading to the reduction of the molecular masses through enzymatic hydrolysis of complex substances (cellulosic and polymeric substances, and high molecular weight organic molecules) and acidophilic reactions with formation of simple
- 30 substances; this stage is characterised by the formation of low molecular weight organic acid (acetic, succinic, propionic acid, etc).

The third stage is defined "methanogenic stage", in that it is based on

the development of a microbial flora which uses the low molecular weight organic acid formed in the previous stage to form biogas.

The relationship between the second and the third reaction stages is of a fundamental importance for the working stability of the process; actually, in the third stage the methanogenic biochemical reactions are characterised by the transformation of short chain acids in methane: said reaction is thermodynamically unfavoured, as in "standard" conditions it has a positive free energy variation (ΔG). Thus, the reaction does not occur spontaneously, and in order to have it proceed the acids level must be kept low; this means that the acids production rate in the acidophilic stage must be compatible with the rate of consumption of the same acid in the methanogenic stage

Therefore a great importance is attached to the bacterial activity to the two cited stages, and to the inhibition factors existing in the substrate. In the frame of the process proposed according to the invention, the proposed technology results from a thorough search on laboratory scale and on a pilot plant, aiming at the following objects:

- isolated and characterising "pools" of microorganisms adapted to the substrate inhibition conditions (e.g. presence of polyphenols);
- individuating the organic load factors per time and volume unit of the reactors;

Individuating the pH conditions and, above all, the temperature conditions of the reactors;

Individuating the hydraulic retention times and the biomass retention times.

d. Results

The biological process results in two substantial variations of the system :

- abatement of the organic load
- biogas production

e. Abatement of the organic load

The abatement of the organic load represents the substrate quantity used for the production of biogas. Such abatement is measured usually

through two parameters:

- variation of volatile solids (SSV);
- variation of COD.

Being the substrate a suspension of OMW/olive husks, it has been
5 considered advisable to measure the abatement in terms of variation of volatile solids.

Said value reached about 60% of the initial value.

f. Biogas production

The produced biogas is a gaseous mixture that is expressed, on average,
10 by the following composition :

	methane	% v/v	65	% w/w	42.50
	hydrogen	% v/v	2	% w/w	0.17
	carbon dioxide	% v/v	30	% w/w	53.91
	ammonia	%v/v	1	% w/w	0.70
15	hydrogen sulphide	%v/v	1	% w/w	1.40
	oxygen	% v/v	1	% w/w	1.32
	density		kg/m ³		1.092
	density relative to air				0.843
	average molecular weight		PM _m		24.47
20	lower calorific value		kcal/nmc		5500

The values reported above may have a wide variability.

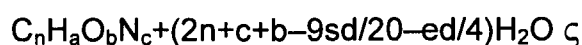
Theoretical model of McCarty

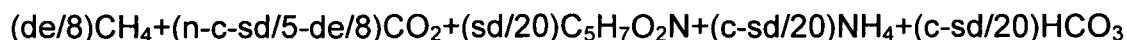
For each substrate undergoing various tests a theoretical yield has
been calculated by applying the McCarty equation starting from the following
25 data:

- elementary composition of the substrate: C, H, O, N
- volatile solids (SSV)

i. Model based on the elementary composition

The McCarty model correlating the biogas production to the elementary
30 composition of the substrate is expressed as follows:





s = COD fraction used for the bacterial synthesis expressed as molar ratio.

e = theoretical conversion fraction from COD to methane expressed as molar ratio (0.5)

5 $d = 4n + a - 2b - 3c$

ii. Model based on COD

the conversion factor is as follows

$$V_{COD, CH_4} = 0.25 \text{ kgCH}_4 / \text{kg degraded COD}$$

iii. Model based on the volatile solids

10 The conversion value of COD / SSV is assumed equal to 1.2-1.5, namely:

$$1 \text{ kg of SSV} = 1.2-1.5 \text{ kg of COD}$$

The foregoing allows to develop the calculation of the biogas productivity in terms of COD.

15 It is assumed that the 60% of SSV loaded in the digester will be transformed into biogas

Applying the provisional models reported above and the experimental data obtained in a pilot plant the following conversion yields have been assumed:

20 **1 kg of SSV eliminated = 0.57 – 0.80 nmc of CH₄**

For a biogas with 65 % methane the following is obtained:

$$\mathbf{1 \text{ kg of SSV eliminated} = 0.87- 1.23 \text{ nmc of biogas}}$$

Referring to the substrates separately and to the following working conditions:

25	- hydraulic retention time	dd	12.5
	- COD abatement	%	66
	- volatile solids abatement	%	60
	- reactor	two stages	
	- pH 1st stage		5.5-6.0
30	- pH 2nd stage		6.9-7.4
	- temperature	°C	30-60

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The following average productivities are obtained:

Olive mill waste water			
	amount	kg	1000
	SSV	kg	39
5	SSV abated	kg	23.4
	Methane produced	nmc	13.3
	Biogas produced	nmc	20.4
	Specific production	nmc CH ₄ /kg SSV ab.	0.57
		nmc biogas/kg SSV ab.	0.90
10		(biogas 65% methane)	

Olive husks amount			
	quantity	kg	1000
	SSV	kg	190
	SSV abated	kg	114
15	Methane produced	nmc	74.1
	Produced Biogas	nmc	114
	Specific production	nmc CH ₄ /kg SSV abb.	0.65
		nmc biogas/kg SSV abb.	1.00
		(biogas 65% methane)	

20 Starting from the McCarty equation the theoretical methane productivities are much higher:

OMW (nmc CH₄/kg SSV ab.) = 0.70 max. value)

Olive husks (nmc CH₄/kg SSV ab.) = 0.80 (max. value).

25 Actually, in the plant working practise, the theoretical values have been approached, and in some experiments values below the average ones have been obtained. Therefore a real productivity range for each substrate may be individuated.

If the following symbols are used:

30 A SSV concentration of the OMW (unitary %)
 B amount of OMW (kg)
 $\alpha = 0.57$ specific productivity of OMW (nmc biogas/kg SSV abated)

- 20 -

C SSV concentration of olive husks (unitary %)
 D olive husks amount (kg)
 $\beta = 0.65$ specific productivity of olive husks (nmc biogas/kg SSV abated)
 (A+ B) total substrate

5 Biogas produced (nmc) = $(A \times B \times 0.6 \times \alpha) + (C \times D \times 0.6 \times \beta)$

The biogas with 65% methane produced will fluctuate by a +/- 50% with respect to the average values

OMW (nmc/methane /kg SSV abated) = 0.285-0.855

Olive husks (nmc/methane/kg SSV abated = 0.325-0.975

10 In the case of a substrate obtained from a two-phase process, and therefore consisting of a mixture of OMW and olive husks, the results do not change in terms of biogas productivity; only the amount of aqueous residue editing the digester is modified

g. Chemical energy-electric power conversion

15 The produced biogas with 65% methane feeds an internal combustion engine for the production of electric power. The following data are assumed:

	- lower calorific value of biogas 65% CH ₄	kcal/nmc	5500
	- conversion yield	% kWe	35
		% kWt	65
20	- electric power	kWh/nmc biogas	2.23
	- thermal energy	kcal/nmc biogas	3575
	Hot water at 90°C:	45% kcal/nmc biogas	2475
	Combustion fumes at 430°C:	15% Kcal/nmc biogas	825
	Loses (attrition ...).	5% kcal/nmc biogas	275

25 The electric power is introduced in the network.

The thermal Energy is used for:

- maintaining the digester at their temperature;
- drying the fluids editing the digester and going to the following treatment for fertilizers production

30 Therefore, the fluids involved in the combustion and produced by it may be characterised in that they are involved in the design of downstream

heat exchangers.

h. Liquid fraction

The fermented substrate from the anaerobic digestion, for the part thereof which is not re-circulated to the process, is filtered on a belt filter press
5 o on a filter press and a solid fraction and a liquid fraction are separated. The liquid fraction contains elements essential to the soil fertility, and in particular it contains carbon and nitrogen in soluble form, mostly of biological origin: this enormously increases the fertilizing value of the product.

The liquid fraction contains the basic elements for fertility:

- 10 - soluble phosphorus
 - soluble potassium
 - soluble microelements.

The liquid fraction is concentrated by means of a reverse osmosis process carried out on tubular membranes. Then, in order to reach the concentration provided for by the legislative Decree of April 26 2006 N. 217 of the
15 Italian Republic a last thermal concentration in evaporator is effected. The final product is as follows:

Organomineral NPK fertilizer: Title 12% % (N+P₂O₅ + K₂O) + 3,0% organic carbon

20 Possibly, additions of substances based on N, P and K may be foreseen in order to reach the title. Reference: Legislative Decree N. 21/2006 paragraph 6.4.1: nitrogen must be at least 2% mineral and 0.3% biological.

i. Solid fraction

A partial reduction of moisture y thermal treatment in evaporator is
25 carried out. The final product is as follows:

Organomineral NPK fertilizer: Title 15% (N+P₂O₅ + K₂O) + 7,5% organic carbon

Possibly, additions of substances based on N, P and K may be foreseen in order to reach the title. Reference: Legislative Decree N. 21/2006
30 paragraph 6.4. Nitrogen must be at least 3% mineral and 1% biological.

Formulation with different concentration of fertilizing unit may be foreseen: this is obtained by varying the concentration process of the liquid or

solid material.

Control analytical techniques for the standardization of the biomass entering the fermentation stage

**1. Method of analysis of the polyphenol compound (Folin Cio-
colteau)**

To 1 ml exactly measured of extract (or solution), 0,25 ml of FOLIN reactive are added, the mixture is left to rest for 3 minutes and then 1,5 ml of Na_2CO_3 AT 20% H_2O is added. The solution is brought to an exact volume of 10 ml with H_2O , it is stirred and left to rest in the dark for 45 minutes; before carrying out the reading the mixture is centrifuged and filtered so as the resulting solution is clear.

Spectrophotometric reading at 725 nm.

The calibration curve, in gallic acid, is shown in the enclosed **Figure 5.**

10 mg in 10 ml in hydro-alcoholic solution are weighted, dilutions are carried out so as to obtain ranges from 10 to 150 $\mu\text{g/ml}$ and the curve is constructed considering that in the procedure dilution is made 10 times.

The spectrophotometric reading of the sample must fall in the linearity interval of the calibration line, so that the starting sample will have to be suitably diluted.

BLANC: the same procedure described above is carried out employing 1 ml of suitable solvent.

2. Method of control of the ortho-diphenol compounds.

1 ml of extract is taken, placed in a test tube for centrifuge and the following is added:

1 ml of reactive A HCl 0.5 N (4.3 ml conc HCl . Brought to 100 ml with distilled H_2O)

1 ml of reactive B: sodium molybdate and sodium nitrite (10 g of $\text{Na}_2\text{MoO}_4 \cdot 2\text{H}_2\text{O}$ + 10g of NaNO_2 dissolved in 100 ml of H_2O)

1 ml of reactive C: NaOH 1N (4 g dissolved in 100 ml of distilled H_2O)

The mixture is stirred and centrifuged for 30 minutes, and then it is read at 500 m.

The enclosed **Figure 6** shows an example of curve with 3,4-dihydroxybenzoic acid.

From the foregoing it is possible to conclude that the proposed system finds an effective placement to the olive mill wastes after the extraction of a polyphenol fraction from the latter. Such extraction, even if it is not so widespread at present, is likely to reach in significant results in time, thus generating highly polluting olive mill wastewater. With the anaerobic digestion treatment and the transformation of digested products in fertilizer of high agronomic value, each fraction either liquid or solid of the waste originated from olive mills is effectively recovered, with a further added value: in addition, biogas produced by the process is recovered, for the electric power production from renewable sources.

The value of the fertilizers in question is described by various agronomy experts who mainly evidence the following aspects:

- the soluble or solubilisable form of the fertilising principles;
- the presence of carbon and nitrogen of a biological origin;
- the presence of organic substance useful for the conservation of the humus in the soil;
- the presence of growth factor (UGF=unknown growth factor) deriving from bioactive peptides, amino acids, vitamins, etc) typical of the starting substrate or that are formed through the fermentation process of the anaerobic digestion.
- the remarkable condition of sanitary safety, in that the substrate derive from thermophilic fermentation.

Many experts of the natural system and of the global impact of the industrial activity on the environment put into evidence, in their turn, the following aspects:

- the production of natural fertilizers results in a slowing down of the use of chemical fertilizers: the synthesis of the latter results in extremely high pollution conditions;
- the use of chemical fertilizers (nitrates, phosphates etc.) has brought about in the years phenomena of water table, water

streams and sea pollution (eutrophization phenomena);

- the use of chemical fertilizers has resulted in a decrement of fertility of the soils, in that they cause an unbalance of the chemical/biochemical composition of the soil (humus depauperation) with a reduction of the reduction yields, so that it is necessary to increase the fertilizers dosages in the course of years.

The invention solves the serious problems of the treatment of the olive mill waste waters, at level of single olive mill or consortia, thus reducing the problems of transportation of huge volumes of wastes, with a reduction of transportation costs and apparent environmental benefits, namely avoiding the spreading on the soils of a phytotoxic industrial effluent, contaminating for the water tables.

To the treatment process according to the invention it will be possible to avoid the release into the environment of a series of chemical substances, such as:

- Simple substances, such as simple perphosphate, ammonium sulphate, potassium sulphate, ammonium nitrate.
- Compounds such as triple granular, mixed of N-P-K;
- Complexes such as calcium nitrate, N-P-K compounds with various concentration;
- Liquids as ortho-phosphoric acid.

The system for the production of natural biofertilizers, liquid and solid, opens a new productive scenarios, also extended to other wastes of the agro-industry. The amounts involved may be relevant so as to substitute a remarkable percentage of the present use of synthesis fertilizers, thus enhancing the reduction of the use of chemistry in agriculture.

In particular some socially relevant aspects deriving from the application of the technology of the present invention are evidenced below:

- A direct help to farmers, in that it makes available an organic fertilizer having an NPK comparable to chemical fertilizers, but having cost at least ten times lower;
- Indirect benefits for the consumers as a consequence of the avail-

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ability of agricultural products treated at "zero chemistry".

It is possible to start a procedure for an ISO 14001 certification of the whole process described above, by means of the application of the LCA (Life Cycle Assessment) method, which is also useful for registering the product obtained
5 both with the Ecolab and "biological" mark , with further environmental innovative quality marks.

The present invention has been disclosed with particular reference to some specific embodiments thereof, but it should be understood that modifications and changes may be made by the persons skilled in the art without de-
10 parting from the scope of the invention as defined in the appended claims.

CLAIMS

1. An integrated process for the production of biogas and energy and integral recovery of chemical compounds and fertilisers from the olive mill wastewaters (OMW) coming from olive oil production wastes obtained through a two-phase process or three-phase process, with possible mixture with olive husks and leafs, comprising the following steps: pre-dilution, centrifugation; selective fractionation for the recovery of polyphenol compounds through a membrane process and adsorption on resins; pretreatment in aerobic and anaerobic attached-biomass columns and tray separation column; anaerobic biodigestion for biogas and fertilisers production; cogeneration for the production of a thermal and electrical power vector; production of water reusable in the process, having hydro-chemical characteristics suitable to civil use.

2. A process according to claim 1, comprising a treatment in aerobic tray separation column (CAS): on exiting the methanogen anaerobic digester (DG3) the substrate is fed to a tray contact and separation column: such column works in aerobic conditions.

3. A process according to claim 1 comprising a treatment in anaerobic attached biomass column (CAN), the column being a multilayer packed column, with the following packing;

- perforated bricks
- sand
- activated carbon.

the function of said inert packing being to offer an anchoring to biomass a thus to create some zones (biofilm) wherein a very high concentration of biomass is present; this allows minimizing the inhibition factors; the active carbon exerting, in addition, a selective adsorption action mainly on enzyme complexes; the bricks performing a more complex function:

- adsorption;
- supply of microelements useful to inorganic catalysis;
- fluid dynamic effect.

4. A process according to claim 1 comprising a treatment of aerobic

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attached biomass column (CAA), wherein the clarified fluid exiting the previous column (CAN) is fed to a combined fluidized bed aeration column, the fluidized bed, having function of biomass fixator, being made of sand and activated carbon.

5 5. A process according to claim 4 wherein the activated carbon performs the following functions:

- it exerts an effective action of biomass accumulation;
- it exerts an adsorption function, which results in a remarkable increase of the retention time of certain macromolecules in the system; said increase in the retention time justifies the global result of increased degradation of certain components which would otherwise be considered resistant;

and wherein the fluid exiting the CAA column reverts to the first stage of the process.

15 6. Use of a pool of microorganisms to be periodically inoculated in the reactors, specifically:

I. methanogen bacteria: isolated from the CAN column medium;
adapted collection microorganisms:

Methanosaeta concihi
Klebisella oxytoca
Clostridium propionicum
Leuconostoc mesenteroides
Clostridium butyricum
Desulfovibrio vulgaris
Methanobacterium formicicum
Metahanosarcina barkeri

II. hydrolytic and hydrogen-productive bacteria: isolated from the DG2 anaerobic digester medium

III. aerobic microorganisms: isolated from the CAA column medium
adapted collection microorganisms

Candida rugosa

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Candida tropicalis
Phanerochete chrysosporium
Pleurotus ostreatus
Laetiporus sulphureus
Pleurotus sajor-caju
Funalia troglia
Paecilomyces variotii
Lentinus tigrinus.

7. A method of standardization of the biomass entering the anaerobic digestion phases by means of monitoring the following parameters:

- Carbon title;
- Nitrogen title;
- 5 ▪ Carbon/nitrogen ratio;
- Total polyphenols (10 % max) and ortho-diphenols (5% max);
- Presence of microelements.

8. A process according to claim 1 comprising a tangential microfiltration (MF) treatment, followed by a tangential ultrafiltration treatment (UF),
10 carried out on the microfiltration permeate, the retentate coming from MF undergoing again pre-dilution and being partly joined to the UF retentate and reprocessed by centrifugation for further cycles of polyphenol extraction and affording commercial fraction of natural antioxidant of polyphenol origin, which are then purified by treatment of polymeric resins and with water, by a treat-
15 ment, which consists in an adsorption-desorption from inert polymeric resins, which may be treated for desorption either with ethanol and with acid water, thereby obtaining from said treatment purified and polyphenol enriched fractions.

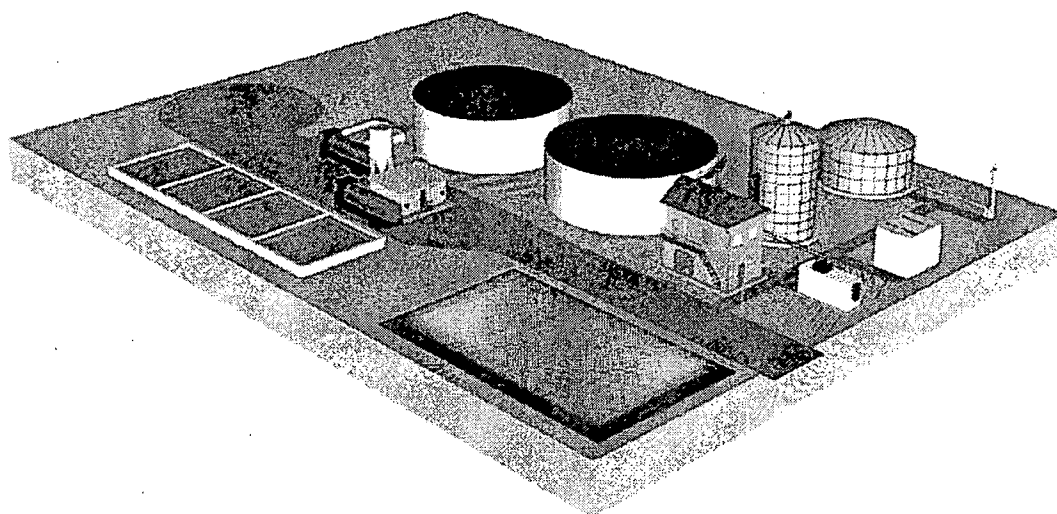


FIG. 1

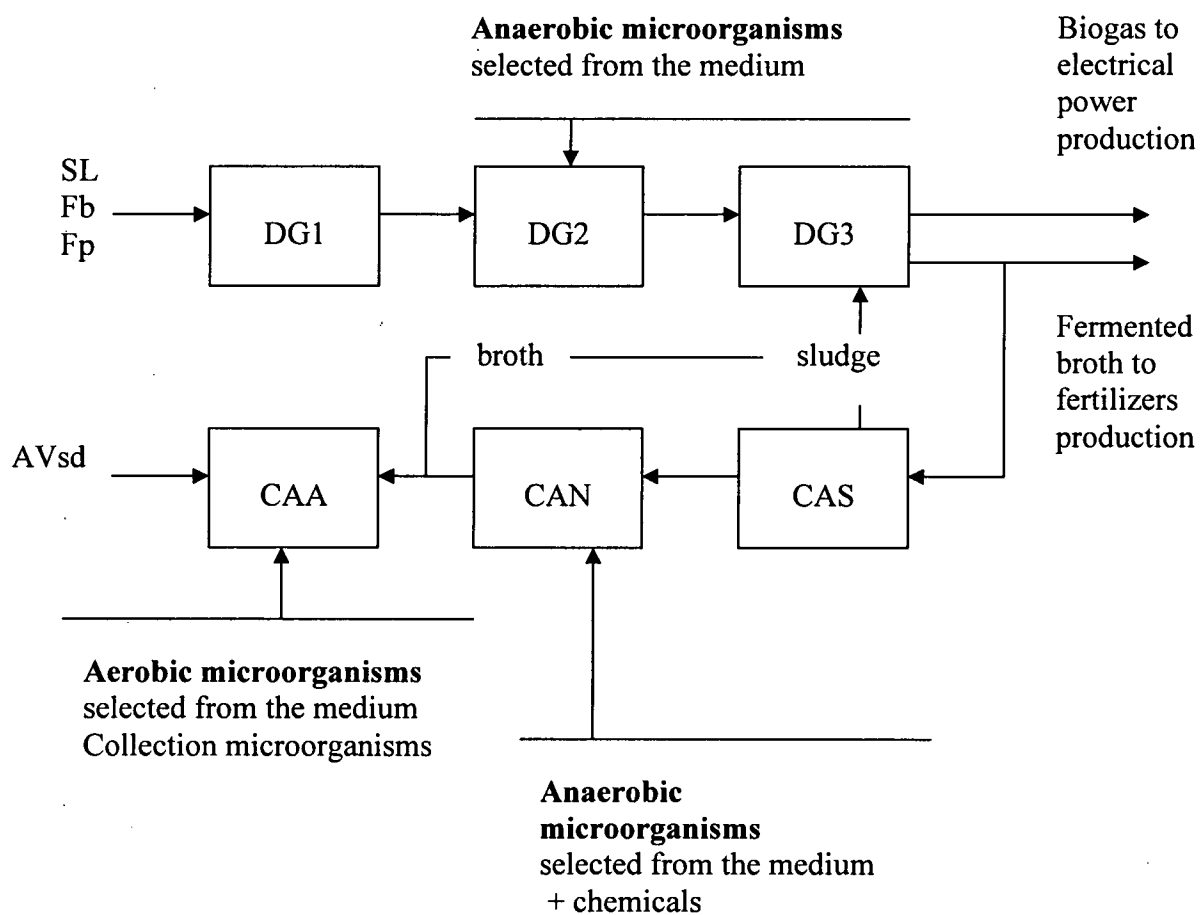
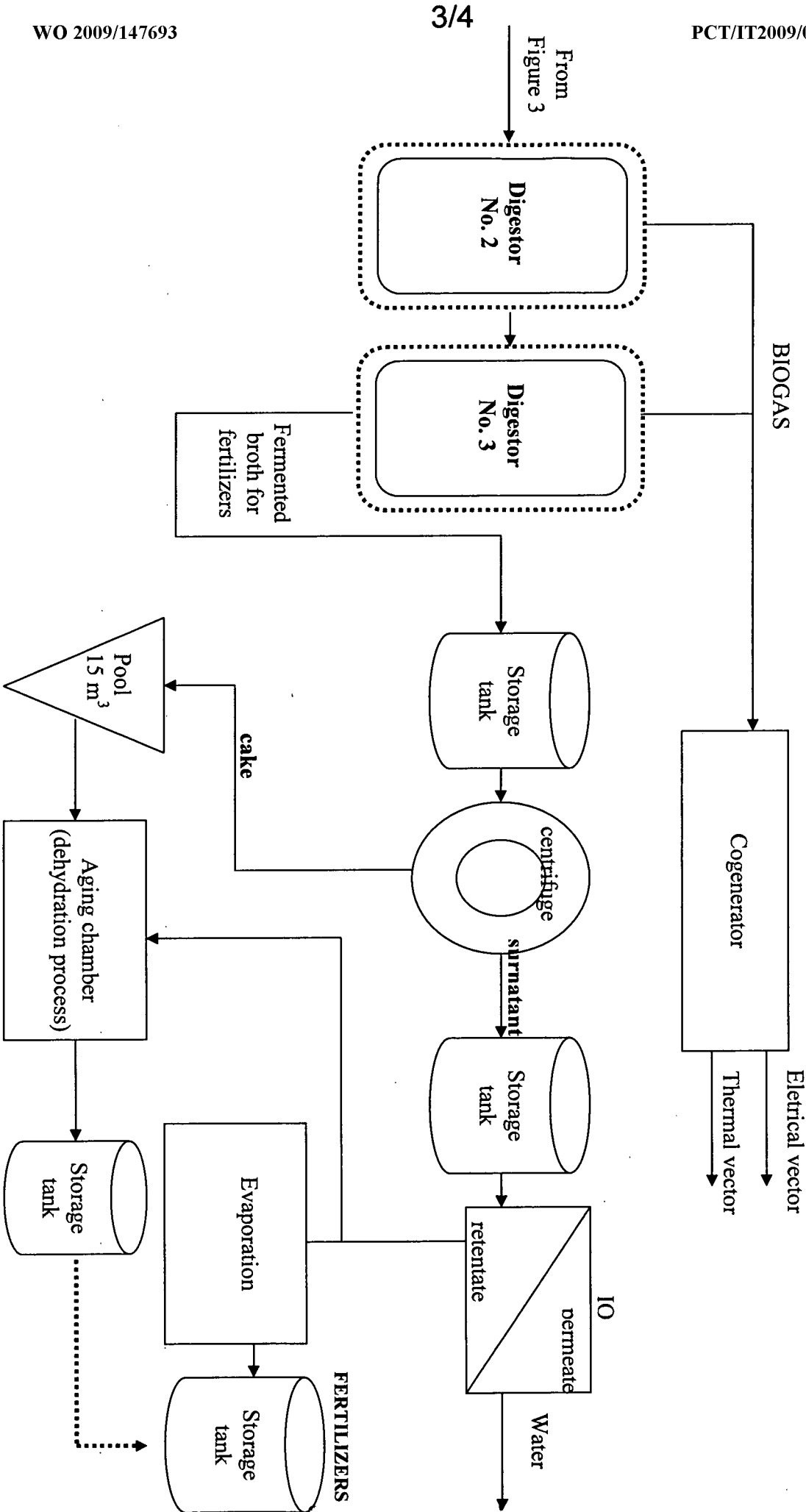


FIG. 2

[illegible]

Fig. 4



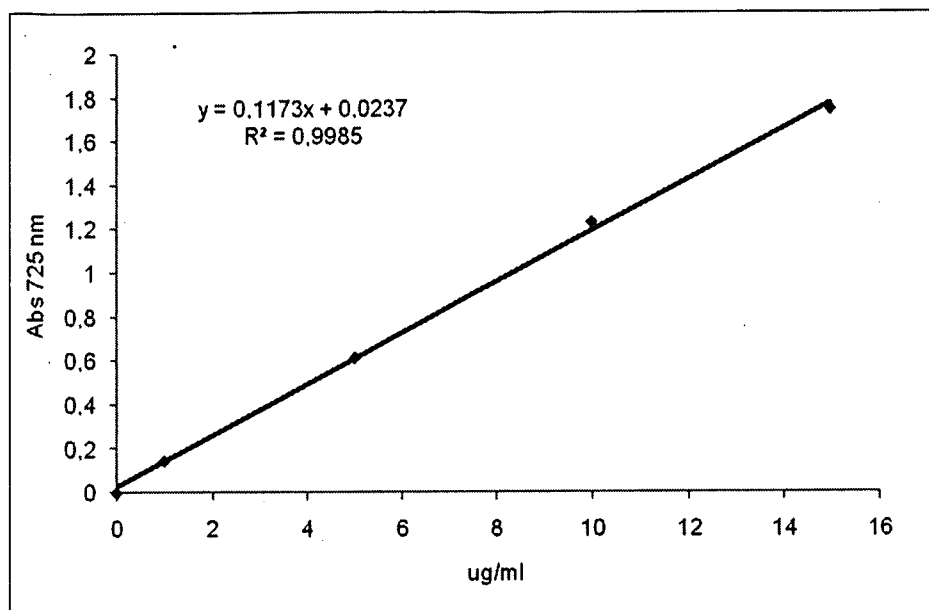


Fig. 5

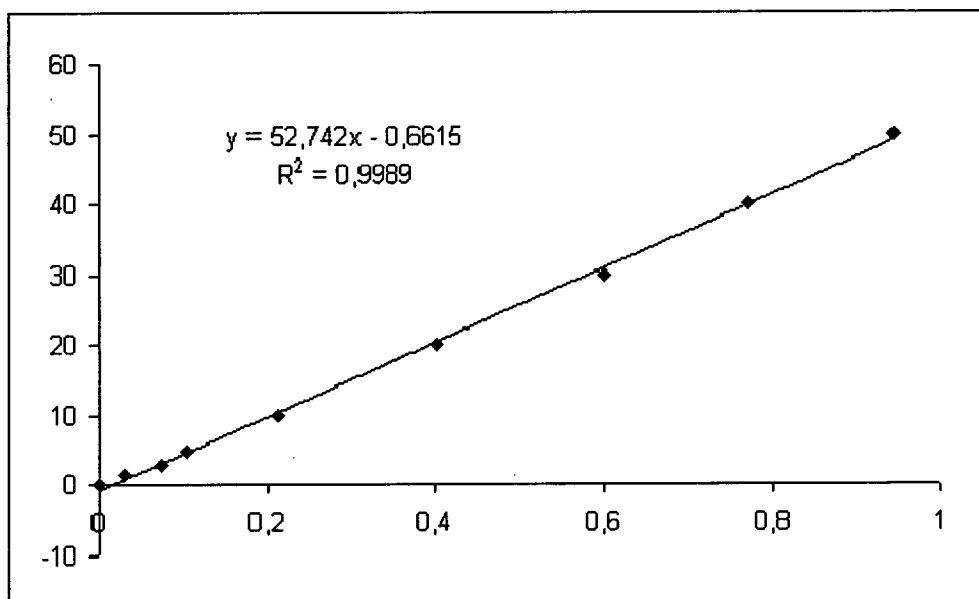


Fig. 6