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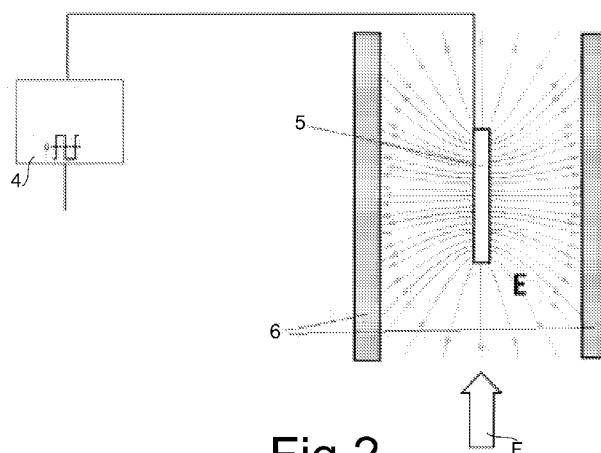


Fig.2

(57) Abstract: An apparatus for treating a fluid (F) containing biological pollutants by means of an electric field (E) comprises an alternating voltage generator (4) that applies in a continuous way an oscillating electric potential to a single electrode (5) connected to a first pole of the generator (4), the other pole remaining unconnected, and operatively insulated from conductive members having an electric potential different from its own, the generator (4) being adjustable at least as to the value and oscillation frequency of the oscillating electric potential applied to the only electrode (5). Through this method it is possible to generate in fluid (F) an electric field (E) such as to cause the irreversible electroporation of the micro-organism(s) to be removed from the fluid without the electrode (5) being crossed by current nor having to resort to high values of voltage applied in pulses, and the relevant apparatus is simpler, cheaper and more flexible in use.



“METHOD AND APPARATUS FOR TREATING FLUIDS CONTAINING
BIOLOGICAL POLLUTANTS WITH AN ELECTRIC FIELD”

The present invention relates to a method and an apparatus for treating
5 fluids containing biological pollutants (viruses, bacteria, fungi, etc.) with an
electric field, typically alimentary fluids such as water, milk, orange juice, beer,
etc. or biological fluids such as blood, serum, plasma, etc. or also medical fluids
such as vaccines and the like. The term “micro-organism” will be used in the
following to indicate any type of biological pollutant present in the fluid to be
10 treated.

It is known that introducing a micro-organism into an electric field may
cause the phenomenon of electroporation, i.e. an increase in the permeability of
the cellular membrane, and said phenomenon is used in molecular biology to
introduce substances inside the cells. Each cell, depending on its size and its
15 morphology, has a first characteristic value of electric field E_s that represents the
minimum threshold below which the membrane is not affected. Beyond said
electric field value the cellular membrane presents characteristics of increased
permeability due to the formation of “pores” on its surface, which remain as long
as the electric field is maintained.

20 The cell also has a second characteristic value of electric field E_r that
represents the maximum threshold beyond which the electroporation phenomenon
is irreversible, because the increase in the number of “pores” breaks down the
cellular membrane and the cell is destroyed. Also this maximum threshold
depends on the type of cell, in particular on its size, and it has been found that the
25 greater the cell the lower the electric field required to reach the irreversible
electroporation.

It is therefore clear that the phenomenon of irreversible electroporation
represents an interesting possibility for “sterilizing” a liquid or eliminating a
particular micro-organism contained therein. For example, in the food industry it
30 would be a valid alternative to conventional thermal treatments such as milk
pasteurization or the sterilization of fruit juices, wine and beer. Using such a non-

thermal treatment would allow to preserve the nutritional and organoleptic features of the alimentary fluids and would significantly reduce the cost of certain industrial processes.

5 However, during the research carried out in the last decades it was found that it is a very difficult task to transfer the technique used for laboratory electroporators to an industrial plant capable of performing a continuous treatment on a fluid flowing at a suitable rate inside treatment chambers without requiring to stop it.

10 In fact, although the limitations of laboratory electroporators (e.g. treating few cells in a vial, "care" after the treatment, repeating the treatment until it is successful) can be overcome by acting on their operating modes, the same criterion can not be applied to an industrial plant where continuity and stability are essential, in addition to an acceptable life and a reasonable cost of the plants.

15 Known experimental devices can be generically described as made up of a treatment chamber containing two electrodes essentially consisting of conductive metal plates, facing each other at a distance of few millimeters, between which the fluid to be treated is placed or passed. A pulsed electric voltage is applied to these electrodes according to the methods that proved to be most effective until now, namely the PEF method (Pulsed Electric Field) or the HIPEF method (High
20 Intensity Pulsed Electric Field) which differ in the intensity of the voltage pulse. In practice, the method consists in repeatedly applying intense yet brief electric fields to the fluids containing the biological pollutants by applying between the electrodes voltages ranging from a few thousands up to tens of thousands volts for a time ranging from fractions of a micro-second to milliseconds.

25 The use of a pulsed electric voltage derives from the fact that the voltage not only generates the electric field that affects the micro-organisms present in the fluid but also generates significant electrochemical phenomena. These phenomena are such that the transformation of a cell present in the fluid being treated may be caused by multiple factors whose contributions are not distinguishable, such as:
30 heat, electric field, passage of ions, passage of electrons, immersion in chemical compounds generated by electrolysis, etc. Even coating the electrodes with

insulating material to prevent the passage of current and to limit the release of electrode particles into the fluid being treated was ineffective, because the electroporation phenomenon occurred very partially and at voltages so high as to make any practical system unfeasible.

5 In order to reduce the impact of the electrochemical phenomena it was then thought to change the application of the voltage between the electrodes, i.e. to send voltage pulses rather than continuously providing a DC/AC voltage between the electrodes. The research was thus directed to finding a suitable compromise between voltage, pulse length, number of pulses, shape of the treatment chamber
10 and electronic device for the generation of pulses.

Obviously, in order to obtain effective results, the shorter the pulse length the higher must be the voltage between the electrodes. Moreover, to be sure to remove substantially all the pollutants it is necessary to increase the value of the electric field up to the maximum threshold of the most resistant micro-organism
15 present in the fluid.

Both these factors imply an increase in the voltage between the electrodes and consequently a situation in which the passage of current between the electrodes is further facilitated and the electronic device that has to provide the electric supply becomes quite complicated and expensive.

20 As diagrammatically illustrated in Fig.1, any known apparatus that intends to use the phenomenon of irreversible (or reversible) electroporation is conceptually based on a single method: the electric field E to which the micro-organism is to be exposed is obtained by generating a difference in electric potential, called voltage hereafter, between two electrodes such that the electrodes
25 themselves acts as the two plates of a capacitor.

The voltage pulse is generated by an electronic device that charges a group of capacitors which are periodically simultaneously discharged on the electrodes that in turn act as a capacitor. The amount of electric charge required to charge the group of capacitors is the power consumption of the device, said group of
30 capacitors being necessary since in order to achieve the required high voltage it is necessary to gather a large amount of electric charges, which will then pass from

one electrode to the other through the fluid depending on the fluid resistivity.

This prior art method and apparatus has therefore a first drawback in generating a passage of current between the electrodes which is the cause of the main negative effects of this technique, such as:

- 5 a) chemical reactions in the fluid and on the electrodes;
- b) consumption of the electrodes with consequent need to replace them;
- c) heating of the electrodes and consequently of the fluid being treated that needs to be refrigerated;
- d) use of a great electric power at a high cost;
- 10 e) problems of contact with biological or alimentary fluids that as such should be in contact with amorphous and biocompatible materials and not with electrodes of conductive material (but the electrodes coated with insulating material make the treatment substantially not feasible).

Furthermore, in order to achieve the irreversible electroporation it is
15 necessary to apply a very high voltage between the electrodes to make up for the very short duration of the electric field. As a consequence, there are various other unsolved problems such as:

- f) a minimum threshold of the applied voltage in the order of thousands of volts since the short duration of the pulse requires that, to achieve the irreversible
20 electroporation of the main pathogen micro-organisms, very high voltage values are usually necessary;
- g) a maximum threshold of the voltage applicable between the two electrodes forming a capacitor, beyond which a destructive electric arc is triggered, which implies that certain types of micro-organisms (e.g. spores or viruses) can not be
25 removed due to the impossibility of reaching the electric field value E_r required according to their characteristics;
- h) the risk of a possible electric arc between the electrodes, which requires a pre-treatment of the fluid by auxiliary control devices to make it perfectly homogenous and without bubbles or micro-bubbles in order to prevent or limit the
30 damages caused by an electric arc;
- i) the cost and complexity of the electronic device that generates and controls the

voltage to be applied between the electrodes, in that it is not simple to generate voltages of tens of thousands of volts for times in the order of micro-seconds and some components have such a short life as to make them barely useful for an industrial process.

5 In addition to the above-mentioned problems, the conventional electroporation method also has the serious drawback of a poor selectivity that on the contrary would be highly desirable for many applications, medical and non-medical, in which it would be extremely useful to be able to perform a targeted removal of micro-organisms with given morphological features. Unfortunately
10 such a control of the selectivity is not possible with the known apparatuses in which the only parameter that allows to control the selectivity is the value of the electric field generated between the electrodes, which allows a "one-way" selectivity based only on exceeding a threshold value.

In fact, as previously mentioned, each type of micro-organism has a
15 characteristic electric field value E_r beyond which the cellular membrane breakdown phenomenon can be triggered, and if the fluid contains two types of cells with two respective different values E_r' and E_r'' (e.g. $E_r' < E_r''$) the selectivity can be achieved only for one type of cell. This is clear from the fact that by generating an electric field such that $E_r' < E_r < E_r''$ only the destruction of
20 the first type of cell will be achieved but by generating an electric field such that $E_r' < E_r'' < E_r$ in addition to the destruction of the second type of cell also the destruction of the first type of cell will be inevitably achieved. Therefore the presently available techniques do not allow to selectively remove from a fluid a micro-organism having a given value E_r'' while maintaining another micro-
25 organism having a value $E_r' < E_r''$.

Therefore the object of the present invention is to provide a method and an apparatus which overcome the above-mentioned drawbacks. This object is achieved by means of a method and an apparatus in which the electric field acting on the micro-organism to cause the irreversible electroporation does not generate
30 a passage of current between the electrodes since there is provided only a single electrode operatively insulated from solid conductive members having an electric

potential different from its electric potential and that therefore may act as ground electrodes or points towards which a continuous stream of electric charges may flow.

5 The main advantage of the method and of the apparatus according to the invention is exactly that of generating an electric field in the fluid being treated without the electrode being crossed by current. As a consequence, all the problems caused by the passage of current mentioned at points a)-e) above are solved or at least made negligible:

- 10 - secondary electrochemical phenomena, e.g. electrolysis, do not take place in the fluid;
- the electrode is not consumed and dispersed in the fluid;
- the electrode does not heat up and therefore it is not necessary to refrigerate either the electrode or the fluid, whereby it is possible to make very thin electrodes that can be used, for example, for catheterisation in the medical field;
- 15 - the electric power consumption of the apparatus is limited to the supply to an alternating voltage generator and said consumption is just a small fraction of the consumption of a conventional PEF system;
- the treatment of biological and alimentary fluids is possible in that, by slightly increasing the peak of the electric potential applied to the electrode, the electrode
- 20 can be coated with a thin layer of biocompatible insulating material obtaining the same electric field that would be obtained without the layer of insulating material.

Another fundamental advantage of this method and apparatus resides in the possibility of generating the electric field through the continuous application of an oscillating electric potential rather than through a pulsed voltage. This results in

25 all the problems caused by the high values of applied voltage mentioned at points f)-i) above being solved or at least made negligible:

- since the fluid is subjected to the constant action of an electric field the irreversible electroporation, with results comparable to those achieved through a conventional PEF apparatus, is achieved with values of the electric potential
- 30 applied to the electrode that are even 50 times lower;
- since there is only one electrode, operatively insulated from other conductive

members having a different electric potential, an electric arc can not occur and therefore there is no theoretical maximum limit of the electric potential that can be applied to the electrode;

- for the same reason there is no need for a pre-treatment of the fluid to prevent the onset of an electric arc;
- the electronic device to generate and control the electric potential to be applied to the electrode is very simple, reliable, inexpensive and does not include critical components prone to wear or deterioration.

Still another important advantage of the present method and apparatus stems from the fact that, since the oscillating electric potential is applied in a continuous way, it is possible to adjust not only the electric field intensity but also another of the parameters that affect the electroporation of a given type of cell, namely the oscillation frequency f of the electric potential applied by the alternating voltage generator (whereas the used waveform is substantially irrelevant).

This implies that, as confirmed by tests carried out by the inventor, when leaving the electric field value E and the duration of the treatment unchanged, the percentage of removal of a micro-organism changes depending on the above-mentioned frequency f with a “saddle” pattern. In other words, within a frequency range there is a frequency value that produces the maximum removal of a given type of cell, and said maximum can be greatly different from the minimum.

This characteristic allows to act on the frequency variable to obtain a “bidirectional” selectivity, i.e., with reference to the previously cited example, even in the case where $E_{r'} < E_{r''} < E_r$ it is possible to find a frequency f such that the percentage of destruction of the first type of cell is much lower than the percentage of destruction of the second type of cell.

These and other advantages and characteristics of the method and of the apparatus according to the present invention will be clear to those skilled in the art from the following detailed description of some embodiments thereof, with reference to the annexed drawings wherein:

Fig.1 is a diagrammatic view of a prior art apparatus;

Fig.2 is a diagrammatic view of a first embodiment of an apparatus

according to the invention; and

Fig.3 is a diagrammatic view of a second embodiment of an apparatus according to the invention.

Referring first to Fig.1 there is seen that, as previously mentioned, in a conventional apparatus the electric field E is obtained by means of a generator 1, for example a DC generator, connected to two electrodes that act as the two plates of a capacitor and are arranged inside a treatment chamber. The two electrodes are essentially formed by conductive metal plates, facing each other at a distance of few millimeters, between which the fluid to be treated, indicated by arrow F , is placed or passed. A first electrode 2 is connected to the positive pole of generator 1 and a second electrode 3 is connected to the negative pole of generator 1, and a pulsed electric voltage is applied to these electrodes 2, 3.

In the diagram of Fig.2 there is seen that the novel aspect of the method and apparatus of the present invention resides in the use of an alternating voltage generator 4 that continuously applies an oscillating electric potential to a single electrode 5 connected to a first pole of generator 4. This single electrode 5 is arranged at a substantially central position in a treatment chamber with walls 6 of insulating material, whereby said electrode 5 is operatively insulated from conductive members having an electric potential different from its own, while the other pole of generator 4 remains unconnected.

In other words, an apparatus according to the present invention is substantially made up of:

- an electronic device that continuously generates an oscillating electric potential which can be adjusted in value and frequency;
- only one electrode connected to a first pole of said electronic device, the other pole of the device remaining unconnected; and
- a treatment chamber inside which the electrode is arranged, operatively insulated from other conductors, and through which the fluid to be treated is passed.

In this way it is possible to create around electrode 5 an electric field E whose effect on the micro-organisms present in fluid F is effectively carried out up to a maximum of 15 millimeters from the surface of electrode 5, typically in

the form of a plate, depending on the value of the electric potential generated by generator 4, on the oscillation frequency, on the used waveform and on the possible coating of electrode 5.

It was experimentally checked that the percentage of removal P increases with an increase in treatment time t, for the same distance d from the electrode, whereby it is possible by increasing time t to significantly decrease the value of the voltage V generated by generator 4 in order to achieve a same percentage of removal P, as shown in the table hereunder relating to the removal of *E.Coli* in physiological saline:

10

P (%)	d (millimeters)	V (volts)	t (seconds)
99	2	600	0,5
99	2	180	10
99	2	40	90

These data illustrate how the method according to the present invention allows to achieve a removal of micro-organisms comparable to the conventional PEF method yet applying a significantly lower electric potential, since even when time t is in the order of the tenths of second voltage V is in the order of the hundreds of volts, and when longer times t in the order of minutes are acceptable voltage V decreases to values in the order of the tens of volts.

The effectiveness of the present method stems from the fact that thanks to the continuous application of the oscillating electric potential the micro-organisms are subjected to the effects of a variable electric field for a time that is some orders of magnitude greater with respect to a typical PEF treatment. The latter provides, for example, a pulse of 6 micro-seconds applied 150 times per second whereby the micro-organism is immersed in an electric field for a total of 900 micro-seconds (i.e. 0,0009 seconds) which correspond to just 0,09% of the treatment time with the present method.

The second embodiment illustrated in Fig.3 shows how, in order to increase the surface of the electrode, it is possible to adopt a structure more similar to a

prior art structure as that illustrated in Fig.1. In fact, in this second embodiment the only electrode is not in a single piece but divided into two plates 5' that are facing each other and arranged along the walls 6 as plates 2, 3 of Fig.1, yet with the fundamental difference that both plates 5' are connected to the same pole of generator 4. In this way the two plates 5' always have the same electric potential and form only one electrode, thus assuring that it is impossible for a passage of current between them to occur.

Obviously, depending on the specific manufacturing needs, the number of elements that make up the only electrode can even be greater than two as long as all the elements are connected to the same pole of the generator, and the shape of the electrode can be different from the simple plate shape illustrated above. For example, the electrode could consist of a pipe of electrically conductive material inside which the fluid to be treated is passed, or of a wire of conductive material located inside a pipe of insulating material.

In such an arrangement the treatment chamber consists of the pipe itself and it is easy to keep the fluid in contact with the electrode for the time required for the treatment, said time being determined by the pipe length and by the flow rate of the fluid (it should be noted that for some applications in the medical field the treatment chamber could be unnecessary being replaced by cellular substrates).

Therefore the method for treating a fluid according to the present invention, in the light of the description above, can be summarized in the following steps:

- a) arranging only one electrode connected to an alternating voltage generator suitable to apply in a continuous way an oscillating electric potential to said only electrode;
- b) leaving unconnected the other generator pole to which the electrode is not connected;
- c) operatively insulating the electrode from other conductive members having an electric potential different from its own so as to prevent the passage of electric current;
- d) setting the value and oscillation frequency of the oscillating electric potential applied to the electrode, preferably with a periodical sinusoidal or square

waveform that is symmetrical with respect to zero, such as to generate an electric field E_r suitable to cause irreversible electroporation of the micro-organism(s) to be removed from the fluid; and

- 5 e) putting the fluid to be treated in contact with the electrode for a preset time not shorter than 0,2 seconds while the electric potential is applied to the electrode.

It should be noted that the method steps listed above do not form a sequence to be rigidly followed, since in practice the “preliminary” steps a)-d) are carried out only once during the apparatus setup phase, while the fluid supply in step e) will usually be continuous and the step length will therefore be a consequence of
10 the flow rate of the fluid.

It is clear that the above-described and illustrated embodiments of the method and apparatus according to the invention are just examples susceptible of various modifications. In particular, although in its simpler embodiment the apparatus includes a device that generates a waveform that is symmetrical with
15 respect to zero, it would also be possible to provide a device that generates a waveform that is not symmetrical with respect to zero, i.e. with a peak positive value different from the peak negative value, or even a waveform that does not cross zero whereby the maximum and minimum peak values have the same sign.

Finally, it is obvious that a plant for treating fluids containing biological
20 pollutants of various types may include a plurality of apparatuses according to the present invention connected in series through connectors of insulating material, so as to achieve the removal of all the pollutants in subsequent treatment steps.

CLAIMS

1. Apparatus for treating a fluid (F) by means of an electric field (E), comprising an alternating voltage generator (4) that applies in a continuous way
5 an oscillating electric potential to an electrode (5) connected to a first pole of said generator (4), characterized in that said electrode (5) is operatively insulated from conductive members having an electric potential different from its own and is the only electrode of the apparatus, the other pole of the generator (4) remaining
10 unconnected, and in that the generator (4) is adjustable at least as to the value and oscillation frequency of said oscillating electric potential applied to said only electrode (5).
2. Apparatus according to claim 1, characterized in that the electrode (5) is arranged inside a treatment chamber with walls (6) of insulating material, preferably at a substantially central position.
- 15 3. Apparatus according to claim 1, characterized in that the electrode (5) consists of two plates (5') facing each other and arranged along the walls (6) of insulating material of a treatment chamber, both said plates (5') being connected to a same pole of the generator (4).
4. Apparatus according to claim 1, characterized in that the electrode (5)
20 consists of a pipe of electrically conductive material inside which the fluid (F) to be treated is passed.
5. Apparatus according to any of the preceding claims, characterized in that the generator (4) is adjustable also as to the position of the waveform with respect to zero and/or to the type of waveform of the oscillating electric potential
25 applied to the electrode (5).
6. Apparatus according to any of the preceding claims, characterized in that the electrode (5) has a coating of biocompatible material.
7. Plant for treating a fluid (F) by means of electric fields (E), characterized in that it includes a plurality of apparatuses according to any of the
30 preceding claims connected in series through connectors of electrically insulating material.

8. Method for treating a fluid (F) by means of an electric field (E), characterized in that it includes the following steps:

- a) arranging only one electrode connected to an alternating voltage generator suitable to apply in a continuous way an oscillating electric potential to said only
5 electrode;
- b) leaving unconnected the other generator pole to which the electrode is not connected;
- c) operatively insulating the electrode from other conductive members having an electric potential different from its own so as to prevent the passage of electric
10 current;
- d) setting the value and oscillation frequency of the oscillating electric potential applied to the electrode such as to generate an electric field suitable to cause the irreversible electroporation of the micro-organism(s) to be removed from the fluid; and
- 15 e) putting the fluid to be treated in contact with the electrode for a preset time not shorter than 0,2 seconds while the electric potential is applied to the electrode.

9. Method according to claim 8, characterized in that the oscillating electric potential is applied to the electrode with a periodical sinusoidal or square waveform that is symmetrical with respect to zero.

- 20 10. Method according to claim 8, characterized in that the oscillating electric potential is applied to the electrode with a periodical waveform and step d) also includes setting the position of said waveform with respect to zero and/or the type of waveform.

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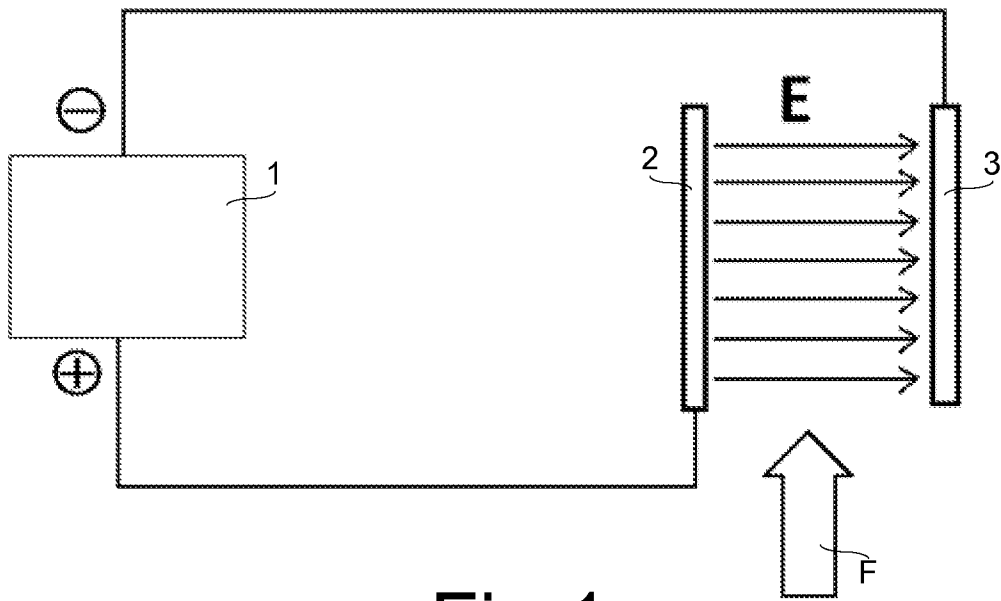


Fig.1

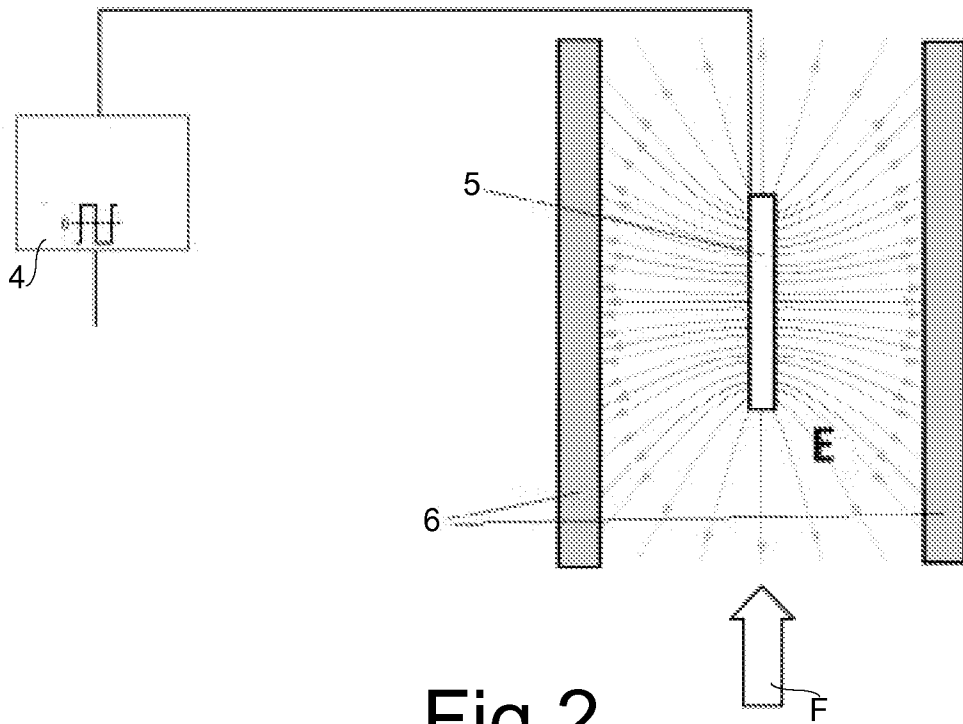


Fig.2

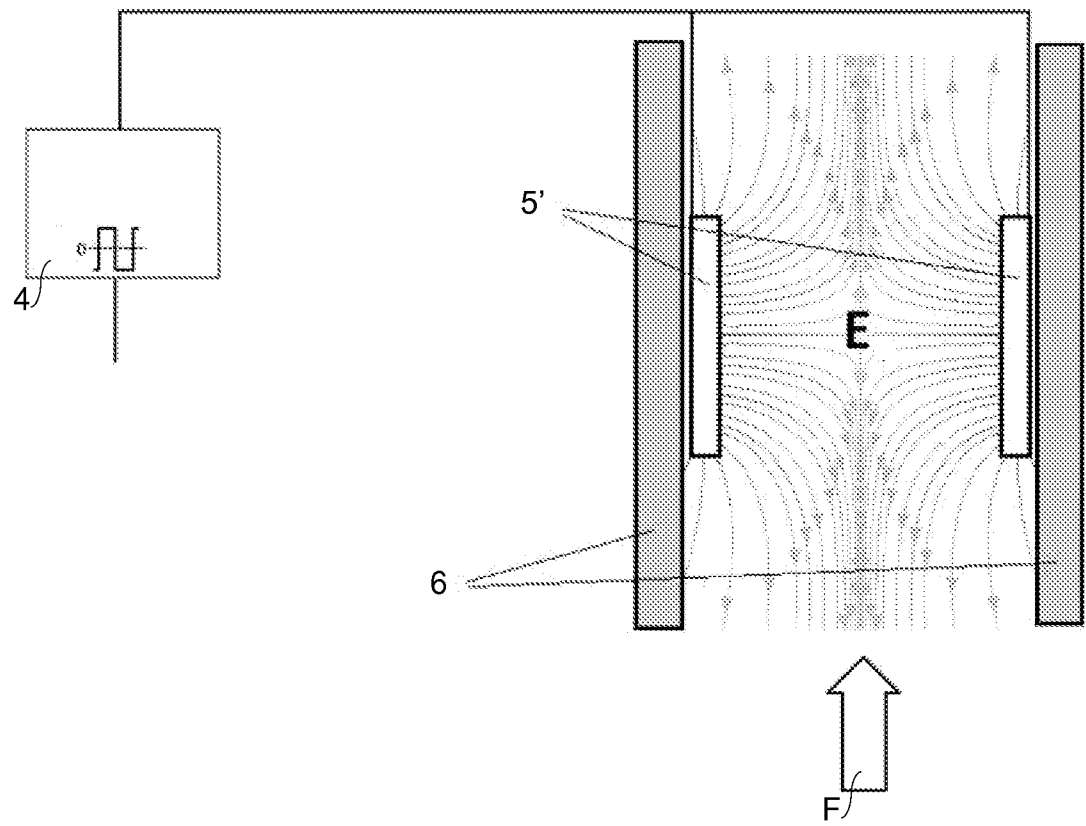


Fig.3

INTERNATIONAL SEARCH REPORT

International application No

PCT/IB2012/051342

A. CLASSIFICATION OF SUBJECT MATTER

INV. A23L1/025 A23L3/32 A23L3/005
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)

A23L

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 practicable, search terms used)

EPO-Internal

C. DOCUMENTS CONSIDERED TO BE RELEVANT

Category*	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
X	WO 03/061409 A1 (YUGENGAISHA BIOFUTURE [JP]) 31 July 2003 (2003-07-31) claims; figure 1 -----	1-10
X	GB 2 433 267 A (MINAGAWA RYUJI [JP]) 20 June 2007 (2007-06-20) claims; figure 1 -----	1-10
X	US 2006/163167 A1 (GODAI TOMOYUKI [JP]) 27 July 2006 (2006-07-27) paragraph [0034]; claims; figure 2 -----	1-10
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A	WO 95/18548 A1 (PUREPULSE TECHNOLOGIES INC [US]) 13 July 1995 (1995-07-13) the whole document -----	1-10



Further documents are listed in the continuation of Box C.



See patent family annex.

* Special categories of cited documents :

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Date of the actual completion of the international search

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INTERNATIONAL SEARCH REPORT

Information on patent family members

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