Abstract

Too many people in the world do not have access to clean and safe water but water purification processes are energy intensive and require structures capable of supplying energy constantly. In many rural areas there is basically no electricity available and only the installation of a photovoltaic system or of any other renewable energy source, can lay the foundations for design of water purification plants. The main goal of this work was to study the effectiveness of ozone (O3) for knocking down high levels of fecal pollution and to construct a prototype for low cost sanitization (O3SU Ozone gas Sanitization Unit), able to supply limited quantities of safe water to small rural communities. Ozone gas generators were tested at 7, 14 and 21 g/h and gas flow times were determined. The ozone sanitization of waters characterized by mainly fecal contamination seems to be very effective; surely the O3SU can and must be improved through, for example, the use of more powerful ozone generators, in order to reduce the duration of the treatment and through the decreasing of the electric consumptions, that would make possible to increase the daily treatment cycles. Thus, we would be able to increase the amount of clean water available to populations living in areas where the water crisis is an unlucky and grim reality.

Keywords: Ozone gas disinfection, Sanitization, Drinking water, Low cost tech, Water safe supply

1. Introduction

“All human beings are born free and equal in dignity and rights” (Universal Declaration of Human Rights, 10 December 1948, art. 1 and preamble). Access to clean water is a fundamental and universal right too, sanctioned for the first time in the history by UN 64/92 of 28 July 2010, and also confirmed by the most recent UN resolution A/RES/70/169 of 17 December 2015, known as “The human rights to safe drinking water and sanitation”.
The "Progress on Drinking Water and Sanitation and Hygiene 2010-2017" Report, published in 2019 and produced within the framework of the JMP (Joint Monitoring Program for Water Supply and Sanitation) of UNICEF and WHO, states that, at the end of 2017, 90% of the world's population, approximately 6.8 billion people, has finally had access to "improved" sources of drinking water.

An improved source of water, whether it is a collection of rainwater, a well, a puddle or a spring, is a source that has been rendered contamination-proof, especially in terms of fecal contamination.

As of now, around 2.1 billion people in the world have no access to clean water and 2.5 billion do not have adequate sanitation.

The cost in term of human lives is very high; according to the UN, 1.4 million people die every year due to diseases caused by contaminated water, mostly children. The worst situation is found in sub Saharan Africa where only 24% of population has access to water and 28% use toilets that are not shared with other families. Furthermore, the UN has also provided data that indicates that water needs have increased by 1% every year since 1980 and many “water wars” have broken out since 2000: precisely 94 wars from 2000 to 2009 and 263 from 2010 to 2018 (UnWater - Leaving no one behind, March 19, 2019). Unfortunately, the goal of access to clean water and adequate sanitation for the whole world population has been postponed to 2030.

Water is drinkable when it is clear, odorless, tasteless, colorless and free of pathogenic microorganisms and chemicals that are harmful to humans; tolerance limits for physical, chemical and microbiological contaminants that are potentially dangerous for our health are established by law.

Water purification processes are energy intensive and require structures capable of supplying energy constantly. In many rural areas there is basically no electricity available.
and only the installation of a photovoltaic system or of any other renewable energy source, can lay the foundations for design of water purification plants, as happened for the AID 10158 / SS / KEN - "Promote access to drinking water and basic sanitation for the population of the Karungu division, Kenya", an initiative of Health and Development NGO, to which ENEA has provided technical support and expertise.

There are several methodologies with which water is made drinkable, but all of them involve expensive equipment or the availability and on-site storage of chemicals, such as chlorine.

The main goal of this work was to study the effectiveness of ozone (O₃) for knocking down high levels of fecal pollution and to construct a prototype for low cost sanitization (O₃SU Ozone gas Sanitization Unit), able to supply limited quantities of safe water to small rural communities.

Ozone gas generators were tested at 7, 14 and 21 g/h and gas flow times were determined for the complete knocking down of the bacterial fecal presence in the polluted waters of the Aniene river, in waters whose bacterial load was predefined in laboratory and in water collected at the end of the filter of Ladispoli, which is characterized by a medium high concentration of fecal origin bacteria. In addition, particular attention was paid to electricity consumption, with the aim of building an autonomous system powered by photovoltaic panels.

2. Materials and Methods

2.1 Water samples

The present study, which lasted five years from 2015 to 2020, is divided into two stages: the first one saw the sanitization tests carried out on 100 liters of water from the Aniene River. The river originates in the Simbruini Mountains, to the N-NE of Rome and flows for 99 kilometers down to Via Salaria, almost in the centre of Rome, where it joins the Tiber. The waters of the Aniene River are characterized, in the lower course, by a high rate of organic pollution, mostly coming from illegal discharges. In this first stage, concentrations of total bacterial load 37 °C (TBL) and total coliforms (TC) have been monitored.

Then, in the second stage, sanitization tests were carried out on 1,000 liters of water from the filter of Ladispoli, a town with over 42 thousand inhabitants, 38 km from Rome, located on the NE coast. The sampling of large quantities of surface water involves considerable difficulties, above all the accessibility to the banks; instead, a water purification plant gives the perfect level of accessibility, equipment and support. In this second stage TC and Escherichia coli (EC) concentrations were monitored.

In both stages, water introduced into the Sanitization Unit O₃SU had preemptively been filtered by way of a homemade system consisting of a small plastic container, some quartz sand and gravel placed in two separate compartments, nonwoven filter and a net
to separate the components; plus an additional filter cartridge for any coarse impurities that might have escaped the first filter (Fig. 1).

![Homemade sand filter and construction scheme](image)

**Figure 1**: Homemade sand filter and construction scheme

### 2.2 Ozone gas

Ozone (from the Greek word “ὀζεῖν” - ozein which means “to smell”) was first used for disinfection (elimination or inactivation of pathogenic microorganisms) between the end of the 19th century and the early 20th century; since then, this method has been used in multiple ways (Rubin, 2001; Gaspari, 2018; Langlis et al, 1991). On June 26, 2001 the FDA - Food and Drug Administration, the US government agency that deals with the management of food and pharmaceutical products, admitted use of ozone in the production processes of the food industry too.

Ozone in the environmental condition (T = 25 °C and P = 1 atm) is a changing gas that rapidly decomposes into molecular oxygen (O₂). It is composed of three oxygen atoms (O₃), it is colorless and characterized a pungent and sharp odor, perceivable in the air even at very low concentrations, 0.04 mg / m³ (~ 0.02 ppm)¹, which have no effect on human health (EPA 1, 1999; Gottschalk et al, 2000).

This olfactory perception threshold of ozone is about five times lower than the maximum concentration allowed in the air in the workplace, or the average hourly occupational exposure limit value MAK (Maximale Arbeitsplatz Konzentrazionen): 0.2 mg/m³ (~ 0.1 ppm) (SUVA, 2006; SUVA, 2014). The Swiss Confederation, in accordance with the Ordinance against Air Pollution (OIAAt), has defined the hourly average limit value for the release of ozone into the air at 0.12 mg/m³ (~ 0.06 ppm).

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¹ 1 ppm of ozone in water corresponds to 1 mg/l, while in air corresponds to 2140 mg/l and this depends on the different density values of water and air
The use of ozone, especially indoors, must always be kept under control by continuously monitoring its concentrations in the air. In fact, it needs close attention because an ozone addiction phenomenon might occur, especially due to the fact that, after a short time, the ozone can no longer be perceived by our sense of smell. Fortunately, at higher concentrations, ozone has a chlorine like odor, much more pungent and annoying.

As the ozone gas concentration increases, the possible harmful effects on people’s health increase too, leading to immediate death (Wunderlin, 2016; LD 13 August 2010, n. 155); table 1 shows the possible health effects and the corresponding hourly average values of ozone gas concentration in the air:

Table 1:

| Ozone gas concentration | Possible health effects                                           |
|-------------------------|-----------------------------------------------------------------|
| > 0,2 mg/m³ (~ 0,1 ppm) | Cough, chronic bronchitis                                       |
| > 1,0 mg/m³ (~0,5 ppm);| Severe eye irritation, nosebleeds and respiratory problems      |
|                         | with severe cough                                               |
| > 2.0 mg/m³ (~ 1.0 ppm)| Chest tightness, dizziness, headaches, circulatory disorders    |
| > 20 mg/m³ (~ 10 ppm)  | Loss of consciousness, hemoptysis, death                        |
| > 10 000 mg/m³ (~ 5 000 ppm) | Immediate death                                                    |

Ozone has a powerful oxidizing action and this feature is widely exploited in wastewater treatment plants and for the disinfection and purification of drinking water for civic use (EPA 1, 1999; Glaze, 1987; Wang and Chen, 2019; Oller et al, 2011; Glaze, 1987). In water, ozone is moderately soluble and it’s a very effective disinfectant because, compared to other reagents commonly used for water treatment, it is the one with the highest standard oxidation potential (E₀) (table 2):

Table 2:

| Reagent             | Formula | Half-reaction                   | E₀ (V) |
|---------------------|---------|---------------------------------|--------|
| Oxygen              | O₂      | O₂ + 4e⁻ + 4H⁺ = 2H₂O          | +1,23  |
| Chlorine            | Cl₂     | Cl₂ + 2e⁻ = 2Cl⁻               | +1,39  |
| hypochlorite        | ClO⁻    | ClO⁻ + 4H⁺ + 5e = Cl⁻ + 2H₂O   | +1,42  |
| Chlorine dioxide    | ClO₂    | ClO₂ + 4H⁺ + 5e⁻ = Cl⁻ + 2H₂O  | +1,50  |
| Permanganate        | MnO₄⁻   | MnO₄⁻ + 4H⁺ + 3e⁻ = MnO₂ + 2H₂O| +1,68  |
| Hydrogen peroxide   | H₂O₂    | H₂O₂ + 2H⁺ + 2e⁻ = O₂ + 2H₂O    | +1,77  |
| Ozone               | O₃      | O₃ + 2H⁺ + 2e⁻ = O₂ + 2H₂O    | +2,07  |

The ozone solubility in water doesn’t respond linearly to Henry's law \[ H = \frac{P_{gas}}{[gas]}, \] for which
the solubility of a gas in a liquid is proportional to the pressure of the gas itself, owing to
the fact that this law is not applicable to those gases that change their chemical structure
in water during transfer. In fact, in water, ozone decomposes very rapidly in its radicals \( \text{O}_2 \bullet \) (superoxide), \( \text{HO}_2 \bullet \) (peroxide anion or hydroperoxide) and \( \text{HO} \bullet \) (hydroxyde), whose \( E_0 \)
is equal to 2.80 V (Glaze, 1987; Tomiyasu et al, 1985; Zahnise and Howard, 1980; Wang
and Chen, 2019).

To calculate the ozone solubility in water, the **Coefficient of solubility** ratio expressed
in mg/l is used, by which *Henry's Law* is defined as follows: \( H = \frac{p_{O_3}}{S} \times C_g \), where \( p_{O_3} \) is the
partial pressure of the ozone, \( C_g \) the concentration of gaseous ozone and \( S \) the solubility
factor, which decreases as the temperature increases and represents the inverse of the
**Henry Hc coefficient** (\( H_c \) is the ratio of gas concentrations in equilibrium to gas dissolved
in the liquid and to the gas above the liquid). To summarize it is possible to write \( H = \frac{p_{O_3}}{S} \times \frac{H_c}{[o_3]_{mg/l}} \), but this formula too does not adequately describe the solubility of ozone
in water, because many factors influence the concentration of ozone in water: the
temperature is one of the main factor, but also the partial pressure and the atmospheric
pressure, the pH and the solute concentrations play an important part. In general, it can
be said that the solubility of ozone in water, and consequently its effectiveness as a
sanitizing, increases proportionally to the decrease in temperature, pH, solutes and
turbidity and to the increase in the concentration of ozone in the air and atmospheric
pressure. Another very important element is the ozone flow mode, the effectiveness of
which increases proportionally to the diffusion pressure of the gas in the liquid mass, to
the resulting turbulence and to the reduction of the size of the gas bubbles, which must
be the smallest possible.

The disinfectant action of ozone depends on its ability to damage cell walls and to
break the double bonds of the carbon chain (C = C) of unsaturated fatty acids and of large
macromolecular components, in particular the peptidoglycan layer that underlies the vital
integrity of bacterial cells, especially Gram negative. Gram positive bacteria have a much
thicker cell wall peptidoglycan layer and are structured in a more complex way, which
makes the action of ozone less rapid but still effective even in the presence of endospores
(Guidelines for the use of ozonized water, 2012).

When it comes to viruses, the action of ozone is directed to the disulfide bridges (S-
S) of the protein component of the capsule and to the genetic material contained therein,
as in the case of poliovirus (Von Gunten, 2003; Cho et al, 2002; Jiang et al, 2019).

The interest in ozone has historically grown also following studies that have
demonstrated its effectiveness on protozoa, very resistant to other oxidants, such as
*Giardia lamblia*, which generally causes abdominal pain, fatty and yellowish diarrhea,
weight loss and dehydration (a pathology that becomes chronic in 30-50% of cases), and
the enormously dangerous *Cryptosporium parvum*, whose main symptom in humans is
profuse and watery diarrhea, while in children it can also cause anorexia and vomiting and
is potentially fatal in cases of immunosuppression, e.g. in conjunction with AIDS (Renneker, 1999; Betancourt and Rose, 2004).

In particular, in 1989 the E.P.A. (Environmental Protection Agency) carried out some research to evaluate the effectiveness of ozone against Giardia lamblia, comparing it with other disinfectants such as chlorine (Cl), chlorine dioxide (ClO₂) and chloroamines (NH₃Cl₇). Ozone proved to be the most powerful, regardless of the water pH (EPA 3, 1999).

The strong oxidizing action of ozone effects the inorganic and organic substances too, present in suspension or dissolved in water; in some cases, water might see a high concentration of iron and manganese in the oxidation state +2, generally complexed with organic molecules. Ozone oxidizes these two elements to hydroxide and oxide respectively (Hübner, 2015; Camel and Bermond, 1998).

Particular attention must be paid when water see the presence of bromides (naturally present in water as Br⁻ anionic form), which can be oxidized by ozone to bromates (BrO₃⁻), harmful to human health, because carcinogenic (EPA 1, 1999; Haruta and Takeyama, 1981).

Ozonization also allows to make the water clearer and eliminate any bad odors; the color of a water depends on the presence of metal ions, such as iron and manganese, and organic substances, such as humic and fulvic acids. Ozone oxidizes metal ions and attacks the carbonyl group (C = O) and phenolic (aromatic compounds with a hydroxyl OH group) of organic acids and any artificial dyes, causing them to precipitate (Siddiqui et al, 1997; Swietlik et al, 2004).

2.3 Comparison with chlorine

Chlorine is the most widely used disinfectant in the treatment of water especially in small poor or rural communities and has always been considered very reliable for obtaining safe water from uncontrolled groundwater, surface watercourses and rainwater. In fact, it effectively kills pathogens and the use of chlorine tablets to disinfect water in places where there is no collective treatment for drinking water is very common. The Safe Water System (SWS) program, which began in the 1990s (https://www.cdc.gov/safewater/), provides for a point-of-use chlorine sanitation system (POU), i.e. water chlorination with a diluted solution of sodium hypochlorite directly into the containers of each individual consumer, adding to the water a cap full of bleach (2 caps for turbid water) in a standard-sized container and waiting 30 minutes before drinking. Where this program has been applied, there has been a reduction in the incidence of diarrhea from 22 to 84%.

Chlorine can be easily produced through a relatively inexpensive industrial process of electrolysis of sodium chloride solutions. However, transport from production sites and storage, which cannot be outdoors but in a closed and protected structure, affect management costs. Another very delicate aspect is the need to "handle it with care" since
its effects on human health can be very dangerous, depending on the amount of chlorine handled and on the duration and frequency of the exposure. In fact, infrequent exposure to low concentrations, generally, does not cause negative effects on human health. However, everyone must be very careful, because even the continuous breathing of small quantities of chlorine for short time can have irritating effects on the skin and eyes and on the respiratory system, causing cough, chest pain and retention of water in the lungs. Obviously, the effects also depend on the health of each individual. Laboratory research has shown that repeated exposure to chlorine can also affect the immune system, blood and heart (Weisel et al, 1999, Zaky et al, 2015; White and Martin, 2010; Komulainen, 2004; Lautenschlager et al, 2013).

Chlorine reacts with other chemicals and, in water, it combines with inorganic material to form chlorine salts and with organic material to form chlorinated organic chemicals.

The discovery that these chlorinated by products had negative effects on human health has determined the need to identify alternative systems for the sanitization of polluted free waters and the increase in the use of less harmful disinfectants (Condie, 2004).

Finally, it is now known that the long term application of the chlorination process contributes to the preferential selection of bacteria potentially dangerous for human health, which can become resistant or relatively insensitive to chlorine, as a consequence manage to find their way to our dining tables through the water distribution networks (Lautenschlager et al, 2013; Roi et al, 2015). Among them, we can mention the ubiquitous Bacillus cereus, which causes of gastrointestinal disorders and food poisoning, the Aeromonas jandei, responsible for wound infections and enteritis, and the Aeromonas sobria, which can cause sepsis and septicemia.

Fortunately, ozone is able to inactivate these microorganisms in an effective, fast and scientifically recognized way (Wanqing et al, 2019; Wani et al, 2015; Hunt and Marilqas, 1996; Kim et al, 1999).

2.4 Ozone gas production

The Sanitization Unit (O₃SU) is powered at 12 V; for its operation there has been the employment of a system for the production of ozone (O₃) built in collaboration with local business realities and consisting of three crown cells with quartz tube by Blue Ocean Environmental Ltd. of 7 g/h each, powered at 220 V by means of an inverter. The three crown cells are placed in parallel to one another, for a maximum flow rate of 21 g/h (Fig. 2); in addition, the system is made of 0,8 cm inner diameter rubber hoses, a 12 V, 60 W, 80 l/min compressor (Fig. 3), a 0.2 Kg/cm² at 15 °C flow meter (Fig. 4), a gel silica salts air dryer or desiccator (Fig. 5) and a microbubble diffuser, which in our case it consists of a flat porous ceramic stone with a diameter of 150 mm (Fig. 6)
Figure 2: Crown cell, quartz tube, functioning scheme and crown cells in parallel in the O₃SU

Figure 3: Compressor 12V

Fig. 4: Flow-meter
The atmospheric air, pushed by compressor, before going through the quartz tube of the crown cell, funnels through a gel silica salts air dryer, to prevent excessive humidity from hindering the production of ozone and to avert, in case of humidity inside the crown cell, the reaction of the ozone with nitrogen oxides, derived from the oxidation of atmospheric nitrogen. This reaction could lead to the formation of nitric acid, which it can cause damage to the crown cell itself.

Each crown cell is equipped with a cooling fan to tackle the high temperatures, since they can get particularly hot during the ozone production; a variable share between 85% and 95% of the electricity is transformed into heat.

The control of the quantity of ozone dissolved in the water was monitored by reading the value of the reduction potential, or redox in mV; the monitoring of the redox potential has a significant importance for assessing the germicidal ability of a disinfectant in water. For that purpose, aPCE-228 pH-meter redox meter was used (Fig. 7).

All the equipment described so far have been stored in two thermoplastic containment boxes and mounted on a transport trolley; figure 8 shows a first prototype of O₃SU.

From an economic point of view, ozone sanitization is very advantageous compared
to chlorination systems because it can be powered by renewable energy sources and it is very easy to manage and maintain; in addition, ozone is produced on site, on demand, and therefore the costs of transporting raw materials and of storing do not affect the local economy.

Figure 7: PCE-228 pH-meter redox meter

Figure 8: First prototype of O3SU

2.5 Microbiological analysis

As far as microbiological analysis is concerned, the use of a technology proposed by MBS-HACCP & ACQUE Easy Test was adopted: a product for the microbiological analysis of liquid or solid food, surfaces and waters; in compliance with the Reference Standards, the
method was subjected to the ISO 16140: 2003 Microbiology of food and animal feeding Stuffs, Protocol for the validation of alternative methods. The method requires the sample that needs to be analyzed to be inserted into a little sterile container with selective culture medium, ready for use, and incubated in a small thermostat. Easy Test uses the "Micro Biological Survey - MBS" method, a colorimetric method developed and patented by the Roma Tre University, based on the measurement of the activity of enzymes catalyzing the oxidoreductases of the primary metabolism and on allowing to establish a correspondence between the measured enzymatic activity and microbial load present in the sample (Traversetti et al, 2017; Arienzo et al, 2015; Sanou et al, 2015; Losito et al, 2017; Gionfriddo et al, 2018).

Little containers are equipped with a post analysis self-sterilization system that allows the safe disposal of the vial. After the sterilization, the microorganisms are inactivated, the reagents are rendered inert and the containers can be safely disposed of as "non-hazardous medical waste" in accordance with Ministerial Decree of 25/5/89, that is, in the same way for expired drugs.

To automate the procedure, an MBS-Multireader Analyzer was used, which allows, through a specific MBS-MR software, the simultaneous analysis of eight samples (Fig. 9).

Figure 9: MBS-Multireader Analyzer, Software and one of the container

2.6 System of photovoltaic panels and accumulators

One of the goals of this work is the realization of an autonomous O₃SU powered by solar energy, able to operate wherever the sun shines. For this reason, particular attention was paid to the electric consumption (power absorbed) of all its components (compressor, crown cells, fans, inverter) in order to calibrate power of the solar panels and supporting accumulators to guarantee the necessary energy to ensure multiple sanitization cycles per day. In O₃SU that has been used so far, the compressor absorbs 60 W, the three crown cells 210 W, the four fans 6 W and the inverter 300 W; therefore, the total electrical absorption is equal to about 580 W. Since the O₃SU works with voltage at 12 V,
accumulators must guarantee 48 - 49 Ah. A truck battery can have a capacity of 225 Ah and can theoretically ensure, in the absence of sunshine, about 3 hours of operation. The solar panels, during the daytime, will have to generate an amount of energy equal to the consumption of the whole system and sufficient to recharge accumulators.

Theoretically, the system is supposed to be able to produce 6.000 liters of clean water per day, using three solar panels of 250 W and two truck batteries of 225 Ah.

2.7 Exploitation phase

Research activities began in the summer of 2015 and continued uninterruptedly until the summer of 2019. During this time, efforts were made to improve the sanitization methodology, especially by working on methods of ozone gas flow, to confirm the results reported in the bibliography and to verify the real reliability of the MBS method in determining the microbial load of the samples. For this purpose, a series of comparative tests were carried out with "classic" methods of microbiological analysis: samples of progressive and serial dilutions of the same E. coli culture were grown on permissive MacConkey agar plates; these plates were then incubated "overnight" at 37 °C, to establish the exact bacterial concentration (CFU, colony forming unit). After that, for further confirmation of the cell concentration, the optical density of the bacterial culture was measured using the spectrophotometer at 600nm (Optical Density OD).

In order to let the ozonated air flow spread out in the column of water contained in a tank, the O3 system must be equipped with an air compressor, with a head resilient enough to withstand and to overcome the pressure of the water column and the pressure drops caused by the characteristics of the rubber hoses, the desiccator and the microbubble flow system. In this specific case, common porous stone diffusers for aquariums were used, but there are other better performing and inexpensive diffusers on the market. It is important for the gas bubbles to be as small as possible because their diffusion into a liquid medium depends on the moment of the contact and the size of the area that is impacted; the diffusion capability will therefore be much greater if the volume of gas is distributed in many small bubbles.

For example, a large bubble with an average diameter of 20 mm has a volume of 4.19 cm³ and a surface of 12.6 cm²; while it is also possible to obtain about 296 bubbles with an average diameter of 3 mm, which would cover a total area of 83.6 cm² and would mean the diffusion of the same quantity of gas in a 6.6 times larger water volume or that the diffusion ability in the same volume of water would be 6.6 times greater. With micro bubbles of 100-500 microns these values become incredibly more advantageous. (Https://scubla.it)

\[
V_{d20mm} = \frac{4}{3} \pi r^3 = \frac{4}{3} \pi r10^3 = 4.186,6 \ mm^3 = 4,19 \ cm^3;
\]
\[
V_{d3mm} = \frac{4}{3} \pi r1,5^3 = 14,13 \ mm^3 = 0,014 \ cm^3; \quad \frac{4,186}{0,01413} = 296 \ bubbles
\]
\[
S_{d20mm} = 4\pi r^2 = 4\pi r10^2 = 1.256 \ mm^2=12,6 \ cm^2; \quad S_{d3mm} = 4\pi r1,5^2 = 28,26 \ mm^2=0,28
\]
\( cm^2 \times 296 = 83.6 \, cm^2 \)

Very small gas bubble diffusers cause pressure drops in the air flow; in the O₃SU a 12 V, 60 W, 80 l/min compressor was used (Fig. 3), capable of supplying a real flow rate, determined with a 0.2 Kg/cm² at 15 °C flow meter (Fig. 4), of 2.200 Nl/h. The difference between the nominal flow rate (4.800 l/h) and the real one depends on the pressure drop (about 0.45 atm), determined with a pressure gauge, caused by the whole system and, above all, by the micro-bubble diffuser.

To precisely calculate the quantity of ozone that flows inside the liquid mass, the next formula was used:

\[
Q_{\text{real}}^{\text{NI}} = \sqrt{\frac{[P(\text{read on the pressure gauge})+1]}{[P(\text{flow meter calibration})+1]} \times \frac{[P(\text{flow meter calibration})+273.15]}{[P(\text{environmental})+273.15]} \times Q_{\text{read on flow meter}}}^{\text{NI}}
\]

Normal Liters per Hour are measured in ATA (absolute atmosphere) = atm +1 at 0 °C

At the same time, in order to define how much ozone is dissolved in water, the variation of the redox potential was monitored. Depending on its origins, water has different physical-chemical characteristics that influence the redox potential and it is for this reason that there are no standard value to refer to. For example, graph 1 shows the potential values of 100 liters of drinking water, taken from a tap connected to the Rome aqueduct and stored into a cylindrical container; the changes it experienced after 15 minutes of ozone flow at different concentrations (7, 14 and 21 g / h) are highlighted below.

**Graph 1:** Redox potential variation as a function of concentration of ozone and temperature in 100 liters of drinking water

The graph 1 shows a regular trend because it relates to pure uncontaminated water, with a pH of 6.8; in fact, the increase in the ozone concentration corresponds to an increase in
the value of the redox potential.

The pH of solution, but also temperature and degree of contamination, influence the measurement of the redox potential.

The Nerst equation, by Walther Nerst, with the following formula:

\[ E = E^0 + \frac{RT}{nF} \ln \frac{I_{i(a_{i,ox})}}{I_{i(a_{i,red})}} \]

where:
- \( E^0 \) is the standard reduction potential
- \( R \) is the universal gas constant, equal to 8,314472 J K\(^{-1}\) mol\(^{-1}\) o 0,082057 L atm mol\(^{-1}\) K\(^{-1}\)
- \( T \) is the absolute temperature in K (T standard 298,15 K)
- \( a_{i,red} \) is the chemical activity of the i-th type of chemical in reduced form, to the right of the arrow in the reduction half-reaction
- \( a_{i,ox} \) is the chemical activity of the i-th type of chemical in reduced form, to the left of the arrow in the reduction half-reaction
- \( v_{red} e v_{ox} \) are the stoichiometric coefficients
- \( n \) is the number of electrons transferred in the half-reaction
- \( F \) is the Faraday constant, equal to 96485,309 C mol\(^{-1}\)

expresses the reduction potential in conditions that are different from the standard hypothetical ones and highlights that \( E \) increases as pH decreases.

Temperature has its importance too, as it is able to speed up or slow down the oxidative action of a disinfectant. As early as 1884 Henry L. Le Chatelier developed an important principle relating to a reaction to equilibrium and the consequences produced by the variation of one of the variables that describe the system, that is to say, "the shift from the equilibrium position that counteracts the effects of reaction"; since oxidation is an exothermic reaction \( a + b \leftrightarrow c + d + \text{heat} \), a drop in temperature will tend to shift the balance to the right and to facilitate the reaction. In a completely indicative and not statistically relevant way, it is possible to observe on graph 1 that the highest values of redox potential are obtained with lower temperatures.

Finally, the degree of water contamination also affects the redox potential: if the water sees low level of pollution caused by oxidizable impurities, such as bacteria and organic materials, a relatively small amount of disinfectant is sufficient to achieve a high redox potential. On the contrary, if the water is heavily polluted it will be necessary to increase the disinfectant effect to reach high levels of redox potential and sanitize it.

To verify the effectiveness of the O₃SU, the "Micro Biological Survey - MBS" method was used.

Several kinds of analysis are available but in the present work we have chosen to monitor, in a first stage, the total bacterial load 37 °C (TBL) and the total Coliforms (TC) and in the second stage, Total Coliforms (TC) and \( Escherichia coli \) (EC).

TBL: the bacterial colonies count (CFU) represents a method of analysis of the general water microbial quality; colonies can be grown at 22 and at 37 °C to assess, respectively, the relative proportions of bacteria naturally present in water and unrelated to fecal pollution and bacteria of human or animal origin. The method detects the presence, in
non specific terms, of bacteria, bacterial spores, fecal microorganisms and natural hosts of aquatic environments, which can alter the organoleptic characteristics of the water or cause to opportunistic infections. An increase in the count of bacterial colonies at 37 °C can represent a signal of pollution (Bianco and Tiberti 2019).

TC: They group various aerobic and optional anaerobic bacterial species, Gram negative, no spore forming bacilli present in the feces of humans and animals; moreover they include other bacterial species capable of living in the environment and surviving and growing in water, normally present in the soil and not necessarily coming from the gastrointestinal tract of warm blooded animals (Bianco and Tiberti 2019).

EC: *Escherichia coli* is a Gram negative enterobacter, rod shaped, naturally found in the bottom end of the humans intestine and that of all the other mammals; they are usually transmitted via fecal-oral route. There are particular pathogenic strains producing toxins that can contaminate food and water and cause serious disturbances to the digestive system, such as dysentery. *Escherichia coli* are, among all coliforms, the best specific indicator of fecal pollution, as they are present in large quantities in men and warm blooded animals feces and unable to reproduce in aquatic environments. In most cases, fecal coliform populations are mainly composed of *E. coli*, whose concentration is therefore the first parameter to be monitored in water monitoring programs. In addition, they are an indirect sign of the presence of *Enterovirus*, which are very dangerous for human health (Bianco and Tiberti 2019).

Among the analyzes available, particular attention should also be paid to *Enterococcus*, bacteria more resistant compared to EC and whose presence is an indication of fecal pollution, and to *Salmonella*, which live in the intestinal tract of humans and warm-blooded animals infected. Both bacteria can migrate to soil and water where they are able to survive several months (Bianco and Tiberti 2019).

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The choice of microorganisms monitored in this study resulted from a series of assessments relating to current legislation (in the specific case in Italy: Legislative Decree 31/2001 and 27/2002, DM 1787/2017) on the drinking water and from the need to understand what the optimal flow times and ozone concentrations are in order to obtain an adequate sanitization of the treated contaminated water.

With reference to the extensive bibliography on the effectiveness of ozone as a water disinfectant in very short flow times (Lawrence and Cappelli, 1977; Legube B and Karpel Vel Leitner, 1999; Han et al, 2002; Wysok et al, 2006; Case et al, 2012), TBL and TC were monitored in the first experimental stage to verify the oxidative efficacy of the ozone on a wide range of microorganisms (for example Gram - and + bacteria) and a 5 g/h ozone generator was used for the sanitization of 100 liters of water from the Aniene river, mixing the gas in the water by way a Venturi ejector. The low concentration of ozone and the reduced flow times had no effect on the bacterial concentration. Subsequently, the gas flow times in the liquid mass were increased and the ozone gas concentration increased to 21 g/h. In addition, to diffuse the gas over a larger area of water, the use of microbubble
diffuser (like those used in aquariums) seemed preferable to the Venturi ejectors.

In the second stage, having verified the poor effectiveness of the system on TBL and knowing that the current legislation on the drinking water does not give relevance to this parameter, TC and EC parameters were monitored, yet always in accordance with the law.

It should be stressed that it would be ideal to monitor the ozone sanitization effect on bacteria causing important epidemics such as cholera and typhoid fever, but the handling of such dangerous bacteria involves specific equipment and structures. On the other hand, *Vibrio cholerea* and *Salmonella enterica enterica* are bacteria Gram negative and it is likely that the sanitizing effect of ozone is similar to that obtained on *Escherichia coli* and coliforms. In any case, the prophylaxis and therapies available for cholera and typhoid fever necessarily require clean water, which is the ultimate aim of our study, and adequate levels of hygiene in order to reduce risk factors significantly (Lozano et al, 2012; Bailey, 2011; Antonelli et al, 2017).

3. Results and Discussion

Numerous analysis cycles have been carried out to verify the effectiveness of the sanitization system, optimizing ozone flow times and methods and its concentrations. Hourly samples of water treated with increasing ozone gas concentrations (7, 14 and 21 g/h) have been analyzed to define the bacterial loads. The results have been encouraging, especially with 21 g/h O₃ concentrations for three hours of treatment; under such conditions, we have recorded the complete knockings down of very high bacterial loads.

The graphs below show some of the countless examples of sanitization tests, exemplary for the kinetics of the reaction.

TBL, due to the nature of its composition and the high CFU/ml values normally concentrated in surface and controlled waters, is the most difficult to completely eliminate. However, by increasing the ozone gas concentration and increasing the gas flow times, the number of CFU/ml tends gradually to zero. Nevertheless, this is true only in a very limited quantity of water (Graph 2).

In the three tests shown in graph 2, the starting CFU/ml values are very high, in the order of $10^6 - 10^7$; by bubbling ozone at a concentration of 7 g/h for three hours, no particular reduction in bacterial load has been appreciated. The situation tends to improve with ozone at 14 g/h; in fact, it has highlighted progressive reductions in the bacterial load and a reduction equal to $10^3$ CFU/ml after the three-hour-long treatment. It gets even better with ozone at 21 g/h, which can cause a complete knocking down of TBL.

Results of the sanitization tests of TC in 100 liters water (Graph 3) are different and more positive; these are Gram negative bacteria, and therefore particularly sensitive to the ozone disinfectant effect. Many of them are of environmental origin and able to survive for a long time in the water. In this case, even the lowest concentration of ozone was able, after three hours of continuous flow, to knock down TC completely.
In the second stage, in order to obtain more quantities of sanitized water, tests were carried out on 1 m³ of water from the filter of Ladispoli (RM); it was necessary to check whether the airflow rate generated by the compressor and the gas micro-bubble diffusion in the liquid mass were adequate to get good ozone circulation in the water column. Furthermore, based on the results obtained so far, only ozone gas concentrations at 21 g/h have been monitored.
**Graph 4:** Concentration change in CFU/ml of TC in 1 m³ of water from the filter of Ladispoli

**Graph 5:** Concentration change in CFU/ml of EC in 1 m³ of water from the filter of Ladispoli
Graph 6: Concentration change in CFU/ml of CT e EC in 1 m³ of the filter of Ladispoli with 14 g/h of ozone for 4h

Graph 7: Concentration change in CFU/ml of CT e EC in 1 m³ of the filter of Ladispoli with 21 g/h of ozone for 3h

The comparison of the results between the ozone sanitizing action on TC in a water volume of 100 liters and that in 1 m³ offers an interesting elements of analysis and shows that: the larger the quantity of water to be treated, the more the chances of survival of the bacteria that easily adapt to water. The bacterial load of TC, although it can significantly decrease, never gets to zero; and this happens in most cases. While in smaller volumes (Graph 2), the knocking down of TC is almost constant.
The effect of sanitization on EC, a type of bacteria of fecal origin unable to multiply in aquatic environments, is different; the ozone disinfectant effect can already be visible after 1 hour of treatment; after three hours at 21 g/h all the replicas recorded a total knocking down of EC (Graph 5).

Graphs 6 and 7 show the comparison of the effects of the ozone gas concentrations (14 and 21 g/h) and the duration of its flow (4 and 3 hours) in 1 m³ of water. In both cases, TC, despite a significant decrease, are never completely knocked down, while EC are definitively eliminated. This means that by increasing the concentration in g/h of ozone, it is possible to reduce the duration of the treatment.

4. Conclusion

The ozone sanitization of waters characterized by mainly fecal contamination seems to be very effective; the advantages achievable with use of ozone can be summarized as follows:

- it allows to avoid, or drastically reduce, the use of chlorine compounds and therefore the formation of toxic organic chlorinated by products;
- it does not cause secondary pollution, in fact ozone, after the oxidation reaction, degrades to molecular oxygen and does not leave harmful residues (except in the case of bromates);
- it does not produce sludge or concentrates;
- it is able to oxidize nitrite;
- it does not add further salinity to the water that needs treatment;
- it avoids corrosive and fermentative phenomena with consequent emissions of bad odors, even in case of water storage in containers used for distribution;
- it has great dosage flexibility and plant simplicity, minimizing management and operating control costs.

Excellent prospects can be glimpsed in the treatment of urban waste water for irrigation purposes for agriculture, because the ozone sanitation is able to improve the general characteristics of the water and increase the biodegradability of the waste.

Surely the O₃SU can and must be improved through, for example, the use of more powerful ozone generators, capable of flowing even 30 g/h of O₃ and operating at 12 V; this would make it possible to avoid the use of inverters and reduce the duration of the treatment. These improvements should lead to an increase of the sanitizing capacity of the O₃SU; in fact, by decreasing the electric consumptions, it would be possible to increase the daily treatment cycles. Thus, we would be able to increase the amount of safe water available to populations living in areas where the water crisis is an unlucky and grim reality.
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References

Antonelli G., Clementi M., Pozzi G. P., Rossolini G. M. (2017) – Typhus fever: Medical microbiology principles, 2ª ed., Rozzano, Ambrosiana Publishing House: 220-222
Arienzo A., Sanou M.S., Guetiya W.R, Losito F., Colizzi V., Antonini G. (2015)- Field Application of the Micro Biological Survey Method for a Simple and Effective Assessment of the Microbiological Quality of Water Sources in Developing Countries, Int J Environ Res Public Health. 12(9):10314-28. DOI: 10.3390/ijerph120910314;
Bailey D. (2011) - Cholera, 1st, New York, Rosen Pub., p. 7, ISBN 978-1-4358-9437-2;
Betancourt W.Q., Rose J.B. (2004) - Drinking water treatment processes for removal of Cryptosporidium and Giardia, Veterinary Parasitology, (126), Issues 1–2: 219-234;
Bianco P.M., Tiberti M. (2019)- The legislation makes water on all sides, Part 2, European Consumers, Acts, Cittaducale (Rieti), January 20;
Camel V. and Bermond A. (1998) - The use of ozone and associated oxidation processes in drinking water treatment, Wat. Res., 32, (11): 3208-3222;
Case P.D., Bird P.S., MSc, Kahler W.A., DClinDent, George R, Walsh L.J., DDSc (2012) - Treatment of Root Canal Biofilms of Enterococcus faecalis with Ozone Gas and Passive Ultrasound Activation, JOE, 38 (4): 523-526;
Cho M., Chung H., Yoon J. (2002) - Effect of pH and importance of ozone initiated radical reactions in inactivating Bacillus subtilis spore, Ozone: Sci. Eng., 24 (2) p. 145;
Condie L.W. (2004) - Toxicological Problems Associated with Chlorine Dioxide, Toxicology, 198, Issues 1–3: 239-248;
EPA (1999) - EPA Guidance Manual, Alternative Disinfectants and Oxidants: 3-1 – 3-52;
EPA (1999) - EPA Guidance Manual, Alternative Disinfectants and Oxidants: 1-1 – 1-19;
Gaspari A. (2018) – “Ozone revolution” in the agri-food sector (“Rivoluzione ozono nell’agroalimentare), CesaRicerche Publisher;
Gionfriddo M., Nicolosi B., Murgia L., Arienzo A, De Gara L., Antonini G. (2018) - Field application of the Micro Biological Survey method for the assessment of the microbiological safety of different water sources in Tanzania, Journal of Public Health in Africa 9 (3):154-158, https://doi:10.4081/jphia.2018.905;
Glaze W., H. (1987) – Drinking-water treatment with ozone, Environ. Sci. Technol., Vol 21, No. 3: 224-230
Gottschalk C., Sauge A., Libra J.A. - Ozonation of water and waste water, 2000;
Guidelines for the use of ozonized water in a veterinary clinical setting (2012), Japan Society for the Medical & Hygienic Use of Ozone, Department of veterinary medicine;
Han Y., Floros J.D., Linton R.H., Nielsen S.S., Nelson P.E. (2002) - Response Surface Modeling for the Inactivation of *Escherichia coli* O157:H7 on Green Peppers (*Capsicum annuum*) by Ozone Gas Treatment, Journal of Food Science, 67 (3): 1188-1193;

Haruta K, Takeyama T. (1981) - Kinetics of Oxidation of Aqueous Bromide Ion by Ozone, The Journal of Physical Chemistry, 85 (16): 2383-2388;

Hübner U., von Gunten U., Jekel M. (2015) - Evaluation of the persistence of transformation products from ozonation of trace organic compounds - A critical review, Water Research, 68: 150-170 http://dx.doi.org/10.1016/j.watres.2014.09.051;

Hunt N.K., Marilqas B.J. (1996) – Kinetics of *Escherichia coli* inactivation with ozone, War. Res. 31 (6): 1355-1362;

Jiang H.J., Chen N., Shen Z.Q., Yin J., Qiu Z.G., Miao J., Yang Z.W., Shj D.Y., Wang H.R., Wag X.W., Li J.W., Yang D., Jin M. (2019) - Inactivation of Poliovirus by Ozone and the impact of Ozone on the Viral Genome, Biomed Environ Sci, 32(5): 324-333

Kim J., Yousef A.E., Dave S. (1999) - Application of ozone for enhancing the microbiological safety and quality of foods: a review, J Food Prot, 62: 1071-1087;

Komulainen H. (2004) - Toxicology 198 (2004) 239–248Experimental cancer studies of chlorinated by-products, Toxicology 198: 239-248;

L. D. 13 August 2010, n. 155, in implementation of the Directive on Quality of Ambient Air and Cleaner Air for Europe no. 50/2008 of 21 May 2008 (D L 13 agosto 2010, n. 155, in recepimento della Direttiva sulla Qualità dell’Aria Ambiente e Aria più Pulita per l’Europa n. 50/2008 del 21 maggio 2010);

Langlis B, Reckhow D.A., Brink D.R. (1991) – Ozone in water treatment, Application and Engineering, Lewis Publishers;

Lautenschlager K., Hwang C., Liu W., Boon N., Köster O., Vrouwenvelder H., Egli T., Hammes F. (2013) - A microbiology-based multi-parametric approach towards assessing biological stability in drinking water distribution networks, Water Research 47 (9): 3015-3025;

Lawrence J., Cappelli F.P. (1977) - Ozone in drinking water treatment: a review, The Science of the Total Environment, 7: 99-108;

Legube B., Karpel Vel Leitner N. (1999) - Catalytic ozonation: a promising advanced oxidation technology for water treatment, Catalysis Today, 53: 61–72;

Losito F., Arienzo A., Somma D., Murgia L., Stalio O., Zuppi P., Rossi E., Antonini G. (2017) - Field application of the Micro Biological Survey method for the assessment of the microbiological safety of different water sources in Horn of Africa and the evaluation of the effectiveness of *Moringa oleifera* in drinking water purification, Journal of Public Health in Africa 8 (679): 23-29, https://doi.org/10.4081/jphia.2017.679;

Lozano R., Naghavi M., Foreman K., Lim S., Shibuya K., Aboyans V., Abraham J., Adair T., Aggarwal R., Ahn S.Y. (2012) - Global and regional mortality from 235 causes of death for 20 age groups in 1990 and 2010: a systematic analysis for the Global Burden of Disease Study 2010, Lancet, 380 (9859): 2095–2128;

Matter from Drinking Water Sources, Wat. Res., 31 (12): 3098-3106;

Oller I., Malato S., Sánchez-Pérez J. (2011) – Combination of advanced oxidation processes and biological treatments for wastewater decontamination – a review, Science of the Total Environment: 4141-4166;
Reneker J.L., Marinas B.J., Owens J.H, Rice E.W. (1999) - Inactivation of Cryptosporidium parvum oocysts with ozone, Wat. Res., Vol. 33, No. 11, pp. 2481-2488;

Roi I.Yu., Klimenko N.A., Zdorovenko G.M., Goncharuk V.V. (2015) - Species Identification of Water Microorganisms Resistant to Chlorine Compounds, Journal of Water Chemistry and Technology 37 (3): 145-150;

Rubin M. B. (2001) - The history of ozone. The Schönbein period, 1839-1868; Bull. Hist. Chem., VOLUME 26, Number 1 (2001), ppgg 40-56;

Sanou S.M., Temgoua E., Guetiya W.R., Arienzo A., Losito F., Fokam J., Onohiol J.F., Djemnang B., Zambou N.F., Russo G., Antonini G., Panà A., Colizzi V. (2015) - Water supply, sanitation and health risks in Douala 5 municipality, Cameroon, Ig Sanita Pubbl. 71(1):21-37;

Siddiqui M.S., Amy G.L., Murphy B.D. (1997) - Ozone Enhanced Removal of Natural Organic SUVA (2006) - Swiss national accident insurance fund;

SUV (2014) - Swiss national accident insurance fund;

Swietlik J., Dabrowska A., Raczyk-Stanislawiak U., Nawrocki J. (2004), Reactivity of natural organic matter fractions with chlorine dioxide and ozone, Water Research, 38: 547–558;

Tomiyasu H., Fukutomi H., Gordon G. (1985) - Kinetics and Mechanism of Ozone Decomposition in Basic Aqueous Solution, American Chemical Society, Vol. 24, No. 19: 2962,2966;

Traversetti L., Losito F., Arienzo A., Stalio O., Antonini G., Scalici M (2017) - Integrating running water monitoring tools with the Micro Biological Survey (MBS) method to improve water quality assessment, Plos One 12 (9): 1-12, https://doi.org/10.1371/journal.pone.0185156;

Von Gunten U. (2003), Ozonation of drinking water: Part I. Oxidation kinetics and product formation, Water Res., 37 (7) pp. 1443-1467;

Wang J., Chen H. (2019) - Catalytic ozonation for water and wastewater treatment: Recent advances and perspective, Science of the Total Environment, http:// DOI: 10.1016/j.scitotenv.2019.135249;

Wani S., Maker J.K., Thompson J.R., Barnes J., Singleton I. (2015) - Effect of Ozone Treatment on Inactivation of Escherichia coli and Listeria sp. on Spinach, agriculture 5: 155-169;

Wanqing D., Wenbiao J., Song C., Xu Z., Changping W., Qijun J., Hui H., Renjie T., Song-Fang H., Qilin W. (2019) - Ozone disinfection of chlorine-resistant bacteria in drinking water, Water Research 160: 339-349;

Weisel C.P., Hekap K., Haimieter P., Klott J.B. (1999) Exposure Estimates to Disinfection By-products of Chlorinated Drinking Water, Environmental Health Perspectives 107 (2): 103-110;

White C.W., Martin J.G. (2010) – Chlorine Gas Inhalation, Human Clinic Evidence of Toxicity and Experience in Animal Models, Proceedings of The American Thoracic Society 7 (4): 257,263;

Wunderlin P. (2016) – Aspetti di sicurezza relative all’impiego di ozono negli impianti di depurazione, VSA-Plattform "Verfahrenstechnik Mikroverunreinigungen";

Wysok B., Uradziñski J., Gomólka-Pawlicka M. (2006) - Ozone as an alternative disinfectant – a review, Polish Journal of Food and Nutrition Sciences, 15/56 (1): 1-9;

Zahnise M. S., Howard C. J. (1980) - Kinetics of the reaction of HO2 with ozone, J. Chern. Phys. 73 (4): 1620-1626

Zaky A., BradleyW.E., Lazrak A., Zafar I., Doran S., Ahmad A., White C.W., Dell’Italia L.J., Matalon S., Ahmad S. (2015) – Chlorine inhalation-induced myocardial depression and failure, Physiological Report 3 (6): 1-9