



Article

# Activated Carbon Impregnated with Elementary Iodine: Applications against Virus- and Bacteria-Related Issues

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**Abstract:** An iodine-doped activated carbon (named IodAC) was developed by adsorbing molecular iodine (I<sub>2</sub>) on commercially available activated carbon (AC). Iodine was selected with the purpose to add its well-known antibacterial and antiviral properties to the AC and in order to produce an innovative material for environmental pathogens control and for healthcare-related applications. The impregnation method achieved the goal of strongly adsorbing iodine on the AC surface, since both volatility and water solubility resulted to be negligible, and therefore it did not affect the stability of the material. An antibacterial test (on *Escherichia coli*) and an antiviral test (on an avian influenza strain) were conducted, showing the effectiveness of IodAC against the pathogens. In addition, IodAC was also compared to slaked lime (a material widely used for disinfection of outdoor spaces and livestock farming areas). The data proved the performance of IodAC against virus and bacteria and also evidenced a more stable and long-lasting disinfecting power of IodAC compared to slaked lime, the later reacting with carbon dioxide and suffering a gradually decrease of its disinfectant power; such drawback does not affect IodAC. Overall, the presented results show that IodAC can be used for a wide range of applications, including as a granular disinfectant for public spaces, for water disinfection, zoonotic diseases countermeasures (e.g., as an animal feed additive for avian influenza control), post-harvest food storage, and sanitization. Its characteristics also indicate its potential to be used for medical treatments, such as for blood, intestinal (for HIV, sepsis, irritable syndrome, ulcerative colitis therapy), and medical supplies (antibacterial bandages, gauze, cotton, etc.) sterilization.

**Keywords:** activated carbon; iodine; adsorption; microbiological properties; infectious disease



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## 1. Introduction

Antiviral and antibacterial substances play a primary role in the prevention of infectious diseases, and their relevance was especially highlighted in the last months due to the SARS-CoV-2 (COVID-19) pandemic [1,2]. Although social distancing and personal protective equipment (such as masks and gloves) can act as passive mechanisms to contain the spread of pathogens, approaches based on active disinfection are anyway necessary to ensure safe environments and to protect the most at-risk groups of people (such as the elderly and young children). Besides, since most infectious diseases in humans originate from animals (zoonoses), disinfection, environmental sanitization, and control measures must be applied not only in urban zones but also in livestock farming areas.

Disinfectants are usually divided into three categories [3]: high-level disinfectants, which kill all pathogenic microorganisms, including mycobacteria, but may not effectively

eliminate spores, intermediate-level disinfectants, which may kill mycobacteria, vegetative bacteria, most viruses, and most fungi but do not necessarily kill bacterial spores, and low-level disinfectants, which may kill most vegetative bacteria but do not reliably kill mycobacteria, fungi, non-lipid viruses and do not eliminate spores.

Iodine is one of the halogen elements, and its molecular form ( $I_2$ ) has a strong antiviral/antibacterial power [4,5]; it is used for various disinfection purposes, including water treatment and medical/healthcare applications, e.g., as a skin antiseptic, and in the forms of iodine tincture or mouthwash as povidone–iodine [6–8]. Its biocidal effectiveness is so well established that iodine is even suitable for the control of biohazards [9,10]. Iodine-based disinfectants belong to the medium-level category (the same in which the commonly used chlorine-based disinfectants fall).

Activated carbon (hereinafter referred to as AC) is a microcrystalline, non-graphitic form of carbon produced from various sources (coconut shell, wood, coal, petroleum, etc.), with a porous structure that has been processed to develop its internal porosity [11]. AC's main feature, indeed, is the presence of pores of various sizes, obtained from physical activation (use of hot gases) or chemical activation processes, which have the property to adsorb various substances; taking advantage of this characteristic, ACs are used for a wide range of applications, including deodorization, medical treatments, pharmaceutical processes (such as isolation and purification of ultra-trace substances, for example, the radioactive diagnostic agent  $^{99m}Tc$ ), and water purification [12–14]. Besides, ACs can be regenerated after each employment, by desorbing the adsorbed substances/contaminants; various methods are available for this purpose, including wet oxidation, solvent regeneration, and thermal regeneration [15].

Even if the primary use of AC is to trap and remove contaminants, its adsorption capability can be also useful to impregnate/tie selected substances on the AC surface, consequently obtaining “doped AC” (modified AC) having additional peculiar properties and suitable for specific applications [16–19]. Therefore, considering the abovementioned attributes of iodine and AC our laboratory developed an “iodine-doped AC” (hereinafter referred to as IodAC), whose virucidal and bactericidal applications were tested and are described in this paper.

The use of antimicrobial active ingredients on the surface of AC-based materials was already tested, and its effectiveness clinically proven in various studies (as an example, AC fibers enriched with silver [20], to be used for bandages, AC enriched with drugs [21], and others [22–27]). IodAC, however, introduces an innovative approach to disinfection, since iodine is less expensive than the agents used in other studies and may be easily prepared.

## 2. Materials and Methods

### 2.1. IodAC Production

IodAC was manufactured through the adsorption of  $I_2$  on AC's surface and pores, achieved by the polyiodide wet treatment impregnation method:  $I_2$  was dissolved in a potassium iodide (KI) or sodium iodide (NaI) aqueous solution to obtain triiodide ions ( $I_3^-$ ), then AC was added to the solution to achieve impregnation/adsorption; lastly, the mixture was filtered, washed with distilled water (to remove unreacted iodine), and dried to yield the final material (IodAC).

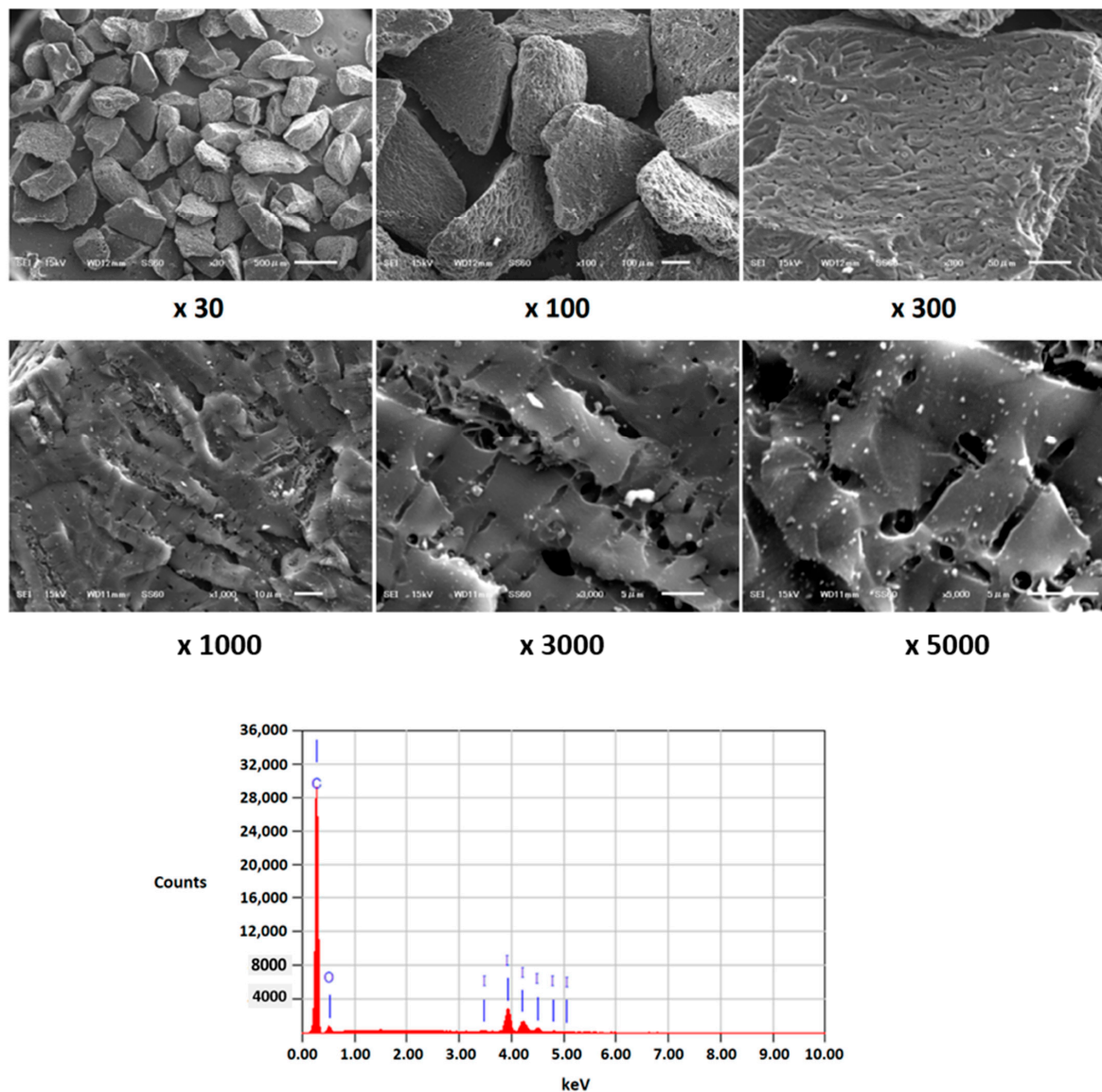
The quantity of iodine adsorbed is directly related to the surface area and pore size of AC. The pores can be micropores (diameter of 2 nm or less), mesopores (diameter of 2–50 nm), and macropores (diameter higher than 50 nm). Before deciding which supporting material to use for IodAC production, various ACs, manufactured from several raw materials, were evaluated, such as coconut-shell AC, having an adsorption capacity up to 1500 mg/g (1500 mg of iodine adsorbed on 1 g of AC), petroleum-based AC, having an adsorption range of 1200–1350 mg/g, wood charcoal AC, having an adsorption of 300–400 mg/g. Adsorption was evaluated by BET surface area analyses and iodine number analyses provided by the manufacturers. The Shirasagi LH2 C-32/60ss, a coconut-shell AC by Osaka Gas Chemicals Co., Ltd. (Osaka, Japan), was finally selected for the production

of our IodAC, since it has a very high adsorption capacity (BET surface area of about  $1500 \text{ m}^2/\text{g}$ .) and a quality/grade ratio suitable for food and pharmaceutical applications. SEM–EDS analysis, performed using a JSM-6510 system (JEOL Ltd., Tokyo, Japan), was carried out both to investigate the morphological features of IodAC and to confirm the presence of iodine on AC surface [28].

Numerous types of IodAC, with different amounts of iodine impregnated on AC, were produced in our laboratory and tested for a wide range of purposes and applications. The experiments hereafter presented in this paper were performed using IodAC (30), a IodAC carrying 30 g of  $\text{I}_2$  per 100 g of product.

## 2.2. IodAC Characterization

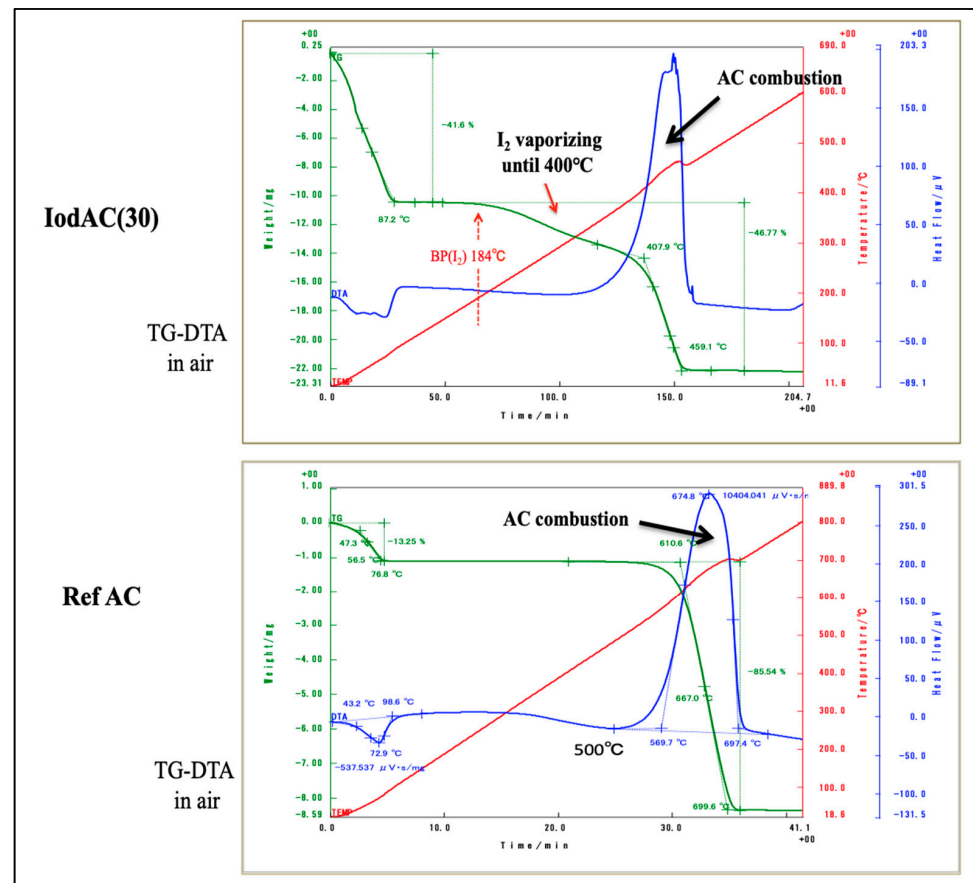
Figure 1 shows the SEM images of IodAC, and the enclosed EDS spectra exhibit the presence of iodine on the surface. The production process (iodine impregnation) did not affect the AC surface, because no superficial differences were observed when comparing IodAC and the raw material (AC before the impregnation) [28].



**Figure 1.** SEM images (various magnifications) and EDS elemental analysis of IodAC (from our paper published in MDPI's *Water* [28]).

Since iodine is strongly adsorbed on the pores' surface, both its volatilization and the elution are negligible (solubility in water  $\leq 0.05\%$  as  $I^-$ ). Besides, although iodine has a peculiar smell, it becomes almost odorless upon adsorption by AC.

The boiling point of iodine ( $I_2$ ) is  $184^\circ\text{C}$ , and it gradually volatilizes from the AC when such temperature is reached or exceeded. However, the residual presence of iodine on AC surface was verified up to about  $400^\circ\text{C}$ , which indicated that the bond was very strong (Figure 2). Instead, practically no desorption/volatilization occurred at temperatures lower than  $184^\circ\text{C}$ .



**Figure 2.** Characterization of IodAC by ThermoGravimetry-Differential Thermal Analysis (TG-DTA) and comparison with raw (not treated with iodine) AC (referred to as “Ref AC”). Analysis performed using a Thermo Plus2 TG8120 (Rigaku, Tokyo, Japan).

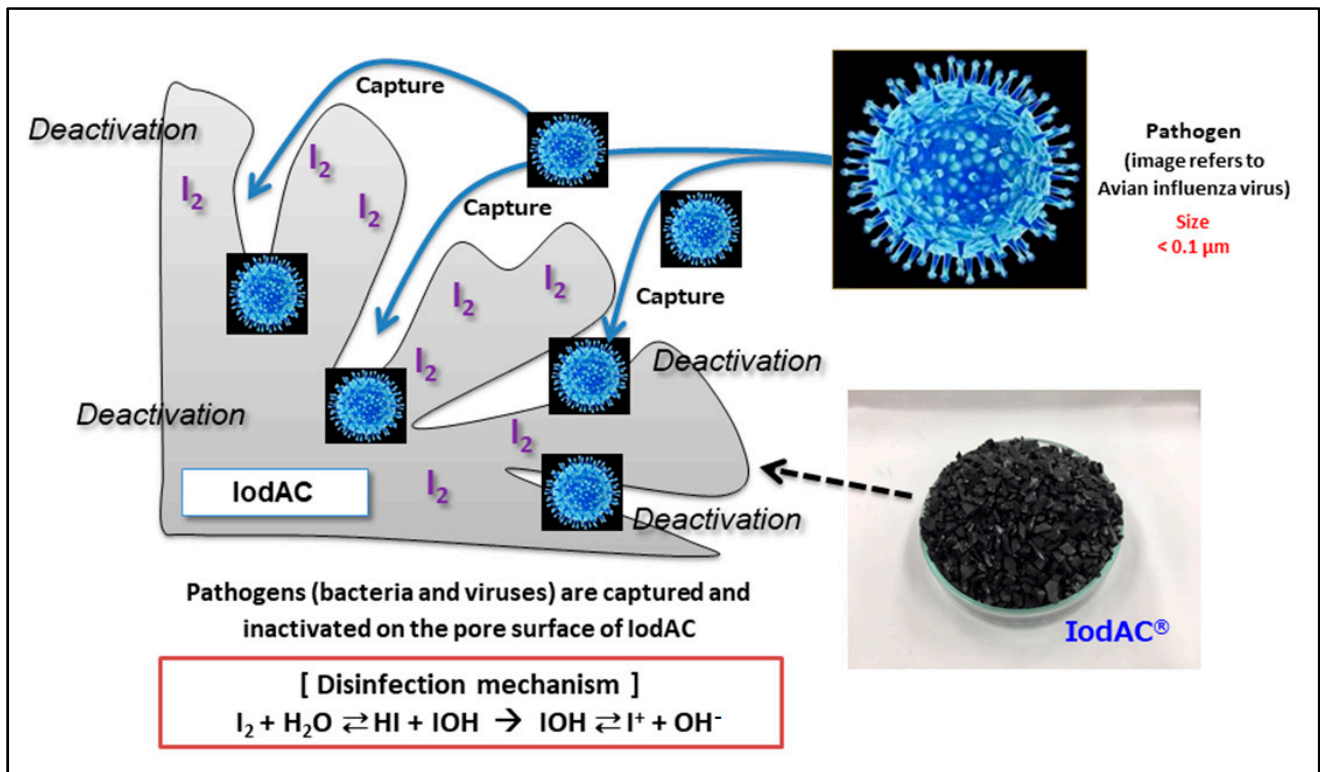
IodAC showed very good endurance against acids, alcohol, and organic solvents at a low concentration (about 2–5%). Low resistance to chemical aggression was instead determined in highly concentrated alkali, alcohol, organic solvents, reducing agents. Furthermore, the aggressiveness of IodAC on other materials was examined. A corrosion test was conducted for about 2 years (700 days), inserting a foil of stainless steel (SUS316) in a vessel, filling the bottom portion of the container up to a few cm with IodAC to cover a portion of the steel, and then filling the remaining volume with seawater. The pH of IodAC was periodically measured, and no change from a value between 4 and 5 was registered. Moreover, at the end of the test, a corrosion rate of only 0.01 mm/y was measured on the stainless-steel foil inserted in the IodAC layer.

A summary of the physical and chemical characteristics of IodAC is reported in Table 1.

**Table 1.** Summary of IodAC, as IodAC (30), main characteristics.

Properties	IodAC
Color, form	Black, granular
Granular size	0.25–0.5 mm
Bulk density	0.50–0.56 g/cc
Element composition	Carbon (C), Iodine (I), Oxygen (O)
Heat resistance	184 °C (I <sub>2</sub> gradually volatile at higher temperature)
Weight loss by drying	≤5.0%
pH	4.0–6.0
Chemical durability	Strong in 2–5% acids, alcohol, organic solvents Weak in highly concentrated alkali, alcohol, organic solvents, reductants
Impregnated iodine amount	I <sub>2</sub> 30 g/AC 100 g
Corrosion rate	<0.01 mmpy (SUS316 in seawater)
Solubility in water	Iodide (I <sup>-</sup> ) < 0.05%; elementary iodine (I <sub>2</sub> ) < 0.05%
Ash	≤2.0%

The disinfecting action of IodAC results from the combination and concurrent presence of stably adsorbed iodine and residual adsorption capacity on the AC surface and pores: indeed, the pathogens (virus and bacteria) are adsorbed and trapped in the pores where they are finally eradicated by the iodine (Figure 3).



**Figure 3.** Explanation of IodAC disinfecting action.

In the case of IodAC (30), consisting of 300 mg of iodine adsorbed on 1 g of AC and produced from an AC having an initial iodine adsorption capacity of 1500 mg/g, about

80% of the adsorption capacity was available for the entrapment of pathogens. Even if only about 20% of the starting adsorption capacity was effectively used to retain iodine, the results presented in this paper show that such an amount is enough to ensure a long-lasting and efficient disinfecting power.

### 2.3. Skin Compatibility and Staining Test

Iodine-based disinfectants (for example povidone) are well recognized as being effective and are daily used by healthcare professionals. However, their main disadvantage is that they can be highly staining on various materials including the skin. In order to check the staining degree, I<sub>2</sub> and IodAC were brought into direct contact with the human palm and wiped off after 1 min. To test for a longer time, a similar experiment was conducted using chicken gizzards, keeping I<sub>2</sub> and IodAC into direct contact with the organ for 8 h.

### 2.4. Antibacterial Test

The antibacterial tests were conducted by preparing three test tubes containing “Brilliant Green Bile Lactose Broth” (BGBLB) medium spiked with a Coliform (*Escherichia coli*) solution: one BGBLB was used as reference (without any addition), one was supplemented with 1 g of AC (raw AC without iodine treatment), and one was supplemented with 1 g of IodAC. The samples were stored at 35 °C for 24 h.

A similar test was conducted employing standard agar culture medium in Petri dishes, spiked with the *Escherichia coli* solution; AC and IodAC were placed on the Petri dish, covering a diameter of about 1 cm on the surface of the agar medium. The dishes were stored at about 35 °C for 22 h.

### 2.5. Antiviral Test

The experiments were conducted using the avian influenza virus “A/whistling swan/Shimane/499/83/(H5N3)”. This is a lowly pathogenic strain isolated in 1983 from the feces of Tundra swans wintering in Shimane Prefecture (Japan) that was confirmed to become highly pathogenic through successive passages in chicks [29,30].

The virus was inoculated in the allantoic cavity of 10-day-old embryonated chicken eggs, cultured at 35 °C for 2 days, then the allantoic fluid was collected and used to prepare the virus solution for the experiments. The 50% Egg Infectious Dose (EID<sub>50</sub>) was calculated and adjusted to about 10<sup>7.5</sup>EID<sub>50</sub>/0.2 mL with sterile Phosphate-Buffered Saline (PBS). The virus solution and IodAC were added to a test tube and mixed for 10 min, testing several IodAC-to-virus solution ratios (such as 1:40, that is, 1cc of IodAC in 40cc of virus solution, or 1:100, etc.) in order to study the variation of the antiviral efficiency. Then, once the mixing was completed, the solution was serially diluted with PBS (1:10, 1:100, 1:1000, etc.), and 0.2 mL of each dilution stage was inoculated into the allantoic cavity of 3 eggs (10-day-old embryonated eggs) and cultured at 35 °C for 2 days. The residual virus titer was calculated as EID<sub>50</sub> using the Reed and Muench method [31].

### 2.6. Use as a Disinfectant for Drinking Water

Disinfection of water destined for human consumption is one of the most important public health-related priorities in any country, since it is strictly related to epidemic prevention. Therefore, evaluating the potential application of IodAC for such a type of purpose is remarkably relevant.

Water disinfection is essentially achieved using continuous treatment systems (for middle-scale and large-scale treatments) or on-demand/portable systems (for individual or domestic-scale treatments). Both types of disinfection were tested.

Continuous-flow experiments were conducted using a 250 cc column, filled with a filter sponge made of IodAC and food-grade polyvinyl acetate (PVA). The AC-based fraction was ground to a size of 6 µm before being combined with PVA. The sponge weighed 14.45 g, and the IodAC content was 40%. The PVA sponge was flexible and perfectly fitted the column. In addition, the use of fine IodAC incorporated in the PVA

matrix assured a better distribution of the AC fraction in the column volume and a better interaction with the water. The disinfection power was tested using an alternating flow of bacteria-contaminated water and sterile water through the IodAC sponge. A glass beaker was filled with 1 L of sterile MilliQ water, which was continuously flowed and recirculated in the column (400 mL/min). The input flow was periodically switched to *Escherichia coli*-contaminated water ( $10^4$ – $10^5$  CFU/mL), which passed through the column at the same flow rate (400 mL/min) for 3 h. The water from the column output was regularly sampled every 15 min (both when the bacteria injection was in progress and when it was not in progress) and was cultured on a standard agar medium, at 37 °C for 18 h, to calculate the bacteria load. Overall, the contaminated water injection was repeated about every 10 days, while the water from the beaker continued to flow without interruption in the column between each injection (to investigate if such prolonged rinsing could induce elution of the iodine component). The flow traveled the column from the bottom inlet against gravity and came out at the top outlet. The experiment was interrupted when the antibacterial power started to decline (that is, when the water sampled from the column outlet was not sterile).

The on-demand (discrete) experiment was performed using a tea filter bag containing IodAC (approximately 1 g, corresponding to 2 cc) and a 500 mL PET (polyethylene terephthalate) bottle filled with *Escherichia coli*-contaminated water (about  $5 \times 10^4$  CFU/mL). After inserting the teabag, the bottle was shaken using an end-over-end shaker, and a water aliquot was sampled every 5 min and cultured (on standard agar medium, at 37 °C for 18 h) to test the residual bacteria load.

### 2.7. Use as a Granular Disinfectant for Public Spaces and Livestock Farming

IodAC was compared to slaked lime, a conventional material widely used as a disinfectant in public spaces (such as after a flood) and livestock farming areas. Commercially available slaked lime and IodAC were scattered in a plastic tray (size 40 cm × 30 cm), at a distribution density of 1000 g/m<sup>2</sup>. Before spreading the two products, a water-absorbing sheet was placed on the bottom of the trays. Every two days, 150 mL of rainwater (previously collected) was nebulized over the tray area. Small amounts of slaked lime and IodAC were collected from the trays every 2–3 days, for about 4 weeks. For each sample collected, pH measurement, antibacterial test (using the test based on *Escherichia coli* as described in Section 2.4), and antiviral test (using the test based on avian influenza virus as described in Section 2.5) were conducted.

## 3. Results

All experiments were repeated three times independently, with similar results. The data, including the values reported in the graphs, are expressed as the mean of the three replicates. The coefficient of variation was always within 5% for all data.

### 3.1. Skin Compatibility and Staining Test Results

The test conducted for one minute on human skin showed that I<sub>2</sub> produced a significant stain, whereas IodAC neither induced pigmentation nor caused irritation (Figure 4). Similar results were obtained from the test conducted on chicken gizzards (Figure 5), confirming IodAC safety for direct and prolonged contact with biological tissues. According to these outcomes, IodAC could be conceivably used as a topical antiseptic and as a material for bandages, gauze pad, or medicated plaster.

### 3.2. Antibacterial Test Results

The results of the antibacterial test conducted using test tubes showed that both the reference and the AC samples were characterized by bacteria growth (the culture media solution became visibly turbid), while the sample supplemented with IodAC remained clear, confirming the strong bactericidal activity of the product (Figure 6a).

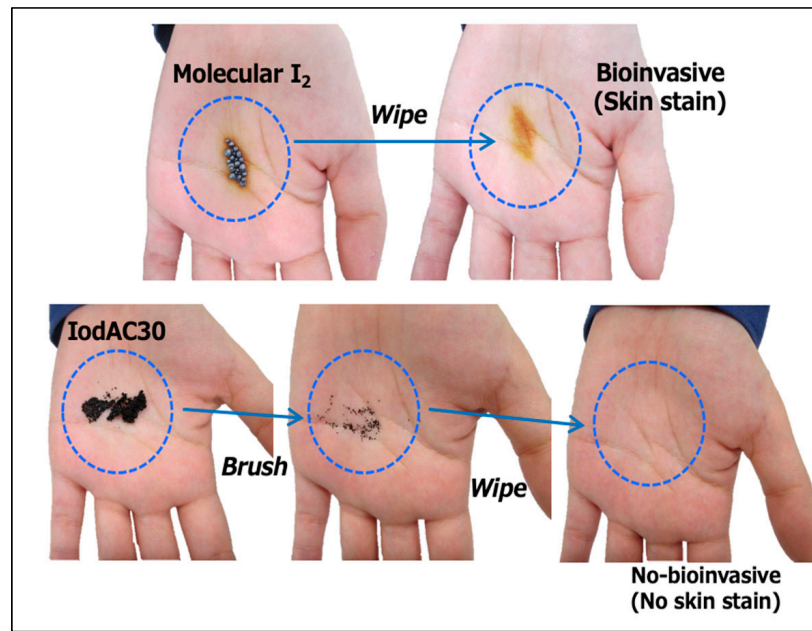


Figure 4. Staining test on human skin, after a one-minute direct contact.

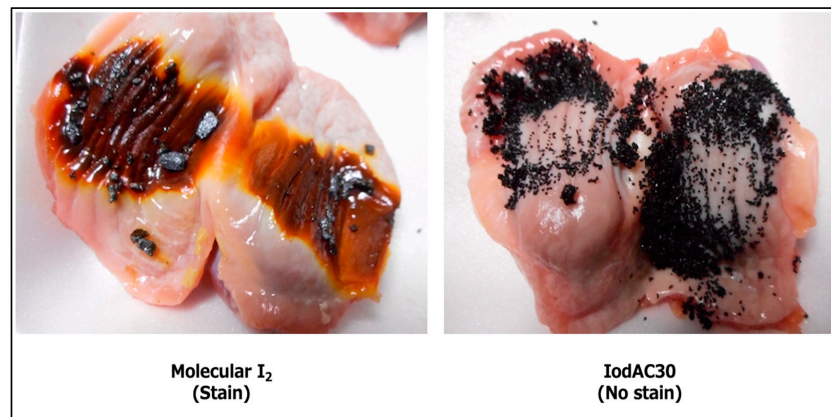


Figure 5. Staining test on chicken gizzards, after direct contact for 8 h.

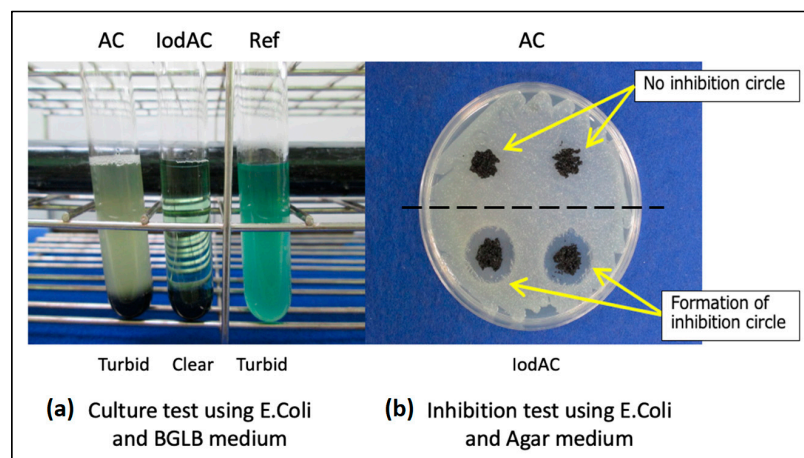


Figure 6. Antibacterial test results: (a) turbidity test for bacterial growth in nutrient broth, (b) zone of inhibition test for antimicrobial activity on agar plate.



In the test conducted on Petri dishes, *Escherichia coli* densely grew around the AC, while a growth inhibition circle was formed around IodAC, confirming again the antibacterial properties of the product (Figure 6b).

### 3.3. Antiviral Test Results

The experiments conducted on avian influenza strain H5N3 confirmed both the suitability of IodAC against viruses and its rapid antiviral effect (Figure 7). After 10 min, even when using a very low amount of product (IodAC/Virus Solution ratio of 1:100), the virus titer was decreased to less than two-thirds of the starting value (represented by the reference sample). Using a IodAC/Virus solution ratio of 1:40, the virus was completely inactivated (with a remaining virus ratio  $< 1/10,000,000$ ).

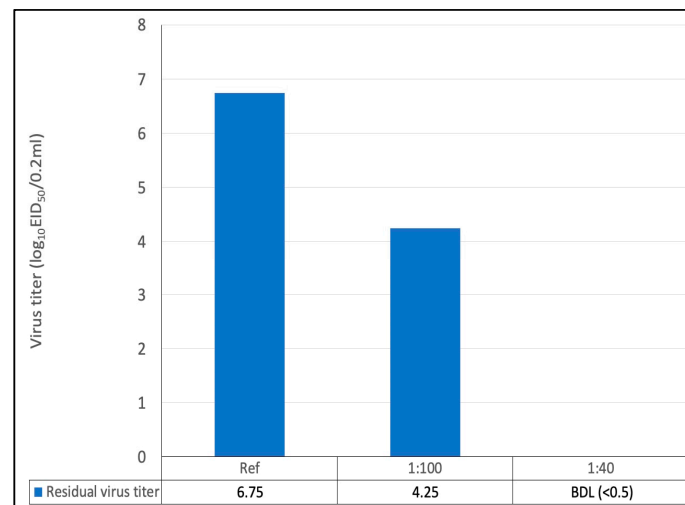


Figure 7. Antiviral test results.

### 3.4. Use as a Disinfectant for Drinking Water

Concerning the continuous-flow test (column test), the results showed that IodAC completely and immediately eradicated the bacteria during the flow in the column. The uninterrupted rinse with water between each bacteria injection showed that elution did not affect IodAC disinfecting features. The antibacterial power started to decline at the end of the third injection, indicating that the disinfecting efficiency of IodAC incorporated in the PVA sponge persisted for over 500 h (over 20 days) of continuous flow (over 48,000 times the volume of the column) and confirming the stable adsorption of iodine on the AC (Figure 8).

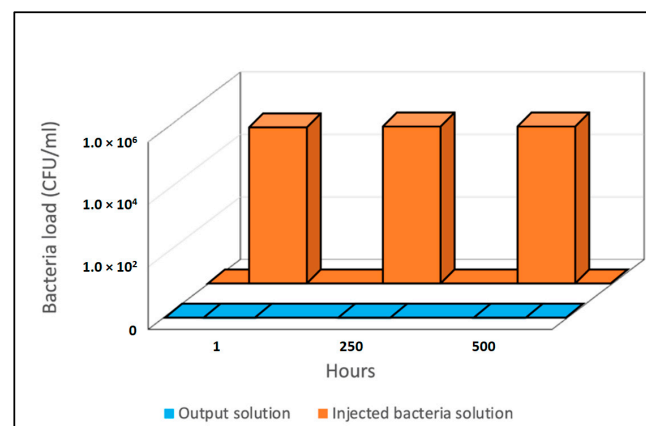
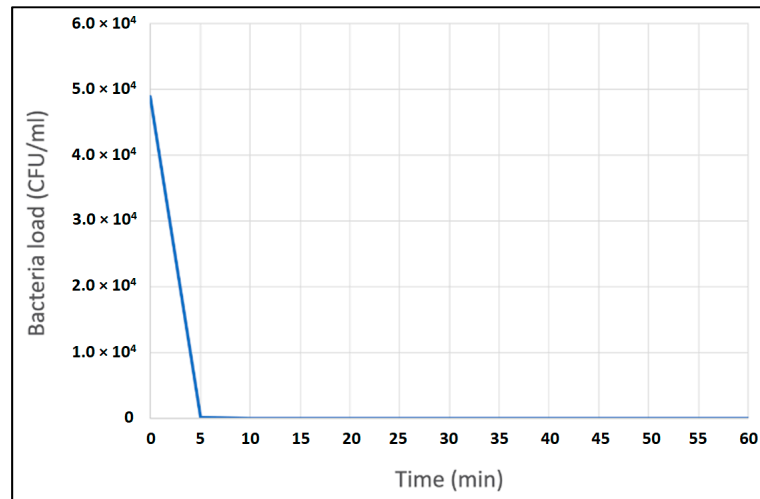


Figure 8. Results of the antibacterial test in the continuous-flow column water disinfection experiment.

The on-demand disinfection test (shaking test) results showed that almost all bacteria were eradicated in the first 5 min of shaking (35 CFU/mL of residual bacteria load), and fully disinfected water (0 CFU/mL of residual bacteria load) was achieved in the next 5 min (Figure 9).

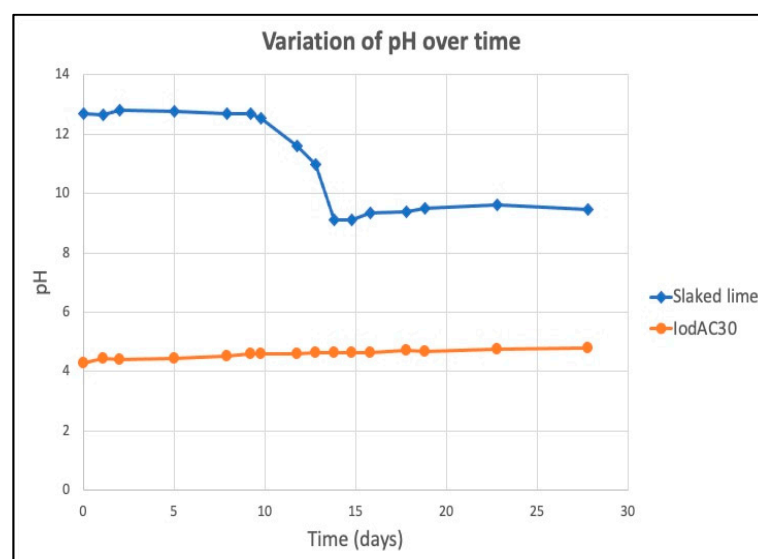


**Figure 9.** Results of the antibacterial test in the on-demand water disinfection experiment.

### 3.5. Use as a Granular Disinfectant for Public Spaces and Livestock Farming

According to all tests performed, IodAC exhibited a more stable and durable disinfecting power than the commonly used slaked lime.

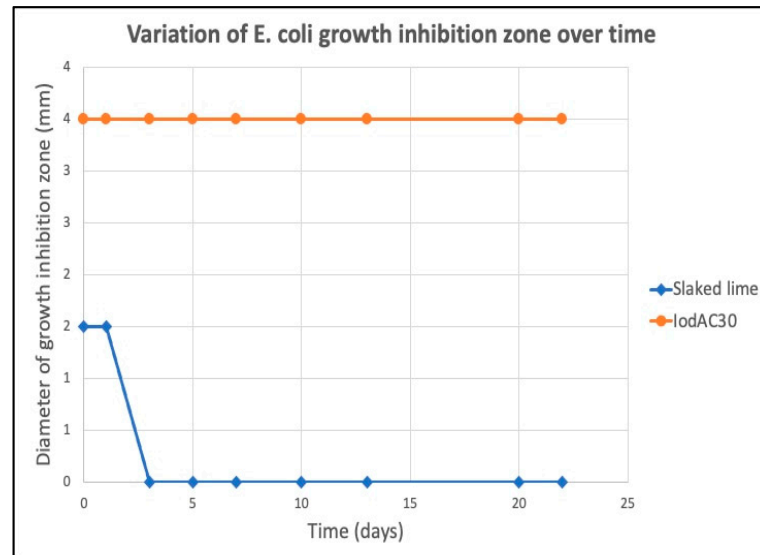
Based on the results shown in Figure 10, the native strong alkaline pH of slaked lime (pH = 12.8) began to intensely decrease after 10 days and stabilized to a pH value of about 9 after the second week. On the contrary, IodAC showed a pH of about 4.3–4.8 during the entire test span (4 weeks). The value of pH is particularly important, since it is strictly correlated to the disinfectant power of slaked lime. Indeed, when exposed to air, slaked lime immediately starts to absorb carbon dioxide, which induces a neutralization reaction that gradually lowers both the pH and the disinfectant power of slaked lime.



**Figure 10.** IodAC vs. slaked lime: variation of pH.

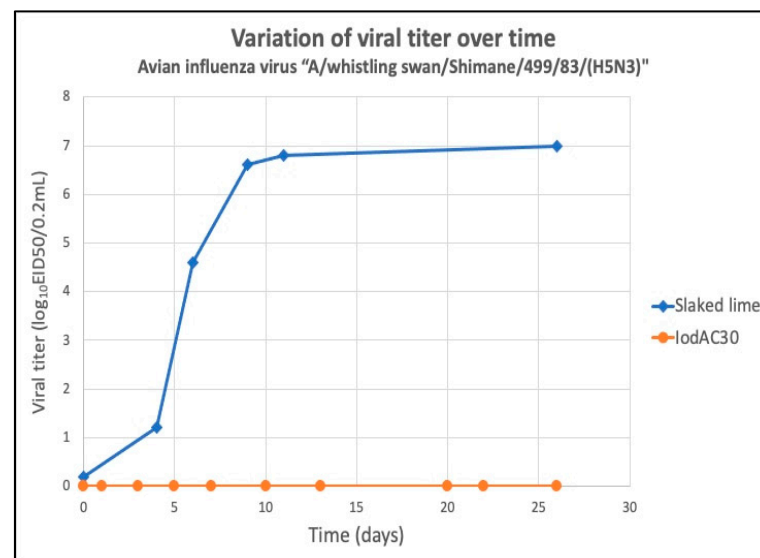
Indeed, as shown in Figure 11, the antibacterial properties of slaked lime, expressed as the diameter (mm) of the inhibition zone, significantly diminished within 1–2 days after

the start of the test and became ineffective in 3 days. IodAC, instead, did not react with the atmosphere and, being characterized by  $I_2$  firmly retained on the AC, showed a stable and high antibacterial activity during the whole test (over 20 days).



**Figure 11.** IodAC vs. slaked lime: variation of antibacterial effectiveness.

Similar results were observed concerning the antiviral test against the avian influenza strain H5N3. As reported in Figure 12, slaked lime was initially efficient, showing a residual viral titer around zero. However, the antiviral power quickly vanished, especially from the 4th day, so that slaked lime became ineffective. Instead, IodAC exhibited again a stable antiviral power, assuring the total inactivation of the virus throughout the test (26 days).

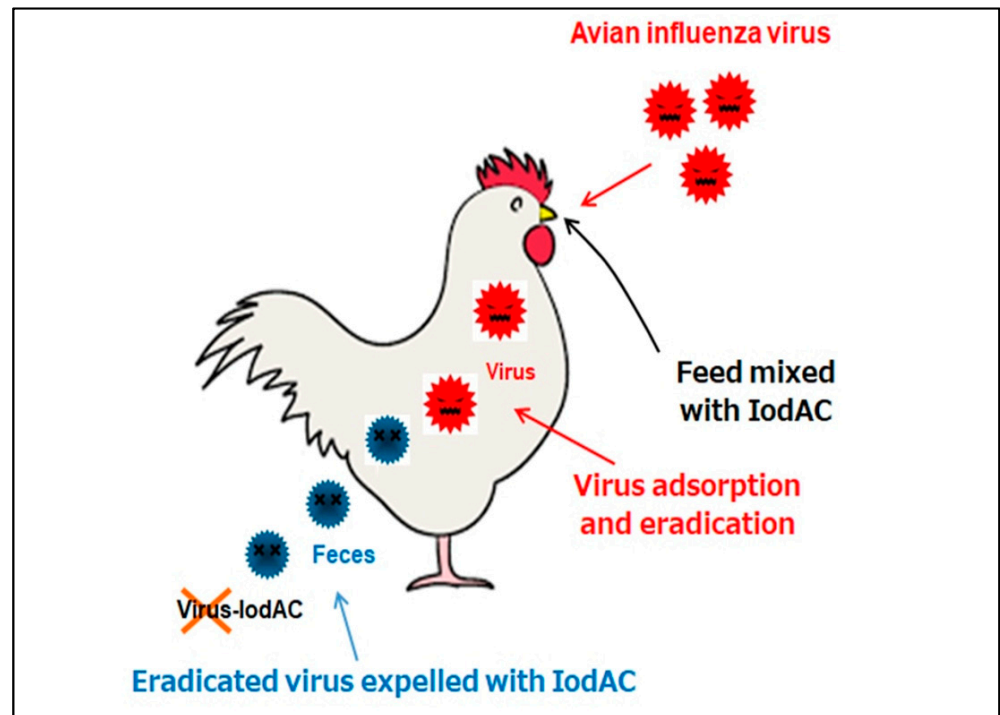


**Figure 12.** IodAC vs. slaked lime: variation of antiviral effectiveness.

### 3.6. Other Application Possibilities

Thanks to its small granular size, IodAC can be useful for many other applications. We already proposed and successfully tested its use in water treatment systems, where IodAC can be used as an oxidant agent in place of sodium hypochlorite (NaOCl) in arsenic removal plants (to maximize the performance of the arsenic sorbent) and as a disinfecting agent [28].

Besides, considering the results presented in this paper, IodAC could be used in breeding farms to inhibit the spread of infectious zoonoses. For example, it could be employed as a feed additive to prevent avian influenza, because animals' feces are a primary route of spread, although the relative amount of pathogen can vary depending on the specific virus, host species, and other factors [32,33]. Therefore, if mixed in proper quantity to poultry feed, IodAC has the potential to inactivate the virus directly inside the intestinal tract of the animal and, since it will not be digested, will be lastly expelled with the uninfected feces (Figure 13).



**Figure 13.** Potential application of IodAC in breeding farms for avian influenza prevention.

Further applications could be chicken eggs wash (Avian influenza/*Campylobacter*/*Salmonella* prevention), blood sterilization treatment/intestinal disinfecting treatment (HIV/Septicemia/Hypersensitivity syndrome/Ulcerative colitis), food conservation (post-harvest citrus management). Moreover, since it is possible to apply ACs to textiles and incorporate them in various forms into permeable materials, IodAC could be also employed as an ingredient for antibacterial/antiviral facial masks (infectious disease prevention), air-conditioner filters, and materials for medical supplies (antiseptic bandage/gauze).

#### 4. Conclusions

Our experiments demonstrated that a long-lasting antibacterial and antiviral power can be imparted to activated carbon (AC) treated with iodine ( $I_2$ ). Iodine, indeed, exhibited a strong affinity for AC, which promoted its stable adsorption on the AC surface and prevented its release in the air or elution in water.

The obtained product, called IodAC, showed a pronounced virucidal and bactericidal activity in various tests conducted against the avian influenza virus and *Escherichia coli*. The results suggest that IodAC can be effective in a wide range of applications in the fields of environmental hygiene, healthcare, and medical technology (both veterinary medicine and human medicine). Some applications were already successfully tested and published [28], and others are currently under testing. Since AC can be incorporated in fibers and textiles, we are also focusing on testing and producing materials enhanced with IodAC.

**Author Contributions:** Conceptualization, K.T.; Data curation, Y.N. and Y.K.; Investigation, Y.K., N.I., T.K., H.K., K.S., T.Y., H.T. and K.O.; Project administration, Y.N. and K.T.; Supervision, Y.N., Y.K., J.F. and K.T.; Writing—Original draft, Y.N. and F.S.; Writing—Review & editing, Y.K. and F.S. All authors have read and agreed to the published version of the manuscript.

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**Institutional Review Board Statement:** Ethical review and approval were waived for this study because the experiments were carried out using poultry embryos.

**Informed Consent Statement:** Informed consent was obtained from all subjects involved in the study.

**Data Availability Statement:** The data that support the findings of this study are available from the authors upon reasonable request.

**Conflicts of Interest:** The authors declare no conflict of interest.

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