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Kinetics with UVC Irradiation Using a Continuous-Flow System: Mathematical Fitting Compared to Microbiological Analysis

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Abstract: Fresh produce contamination poses a significant public health risk. Traditional disinfection methods using chemical solutions, while effective, raise environmental and health concerns. This study explores UVC irradiation, a promising non-chemical alternative proven to be effective against a broad spectrum of microorganisms. We investigated the optimal UVC dosage for reducing microorganisms on fresh vegetables washed in water. Our findings suggest that dosages of approximately 2 mJ/cm^2 in water and 9 mJ/cm^2 in vegetables achieve reductions of up to 99%. Additionally, we established a nominal radiation application rate of 2.38 mW/cm^2/s, reflecting the treatment intensity. Understanding the fundamental mechanisms of UVC irradiation and its interactions with microorganisms is crucial. Elucidating these mechanisms can significantly improve optimization efforts and seamlessly integrate UVC irradiation into food safety protocols. Implementing this strategy offers immense potential to elevate food safety standards in the industry while minimizing environmental impact. This approach aligns perfectly with sustainability objectives by providing a chemical-free solution for food disinfection.

Keywords: UVC; irradiation; foods; decontamination; flows

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1. Introduction

The escalating global concern over population dynamics highlights the urgent need for sufficient food production and equitable distribution. As the world's population grows, demand for processed food increases, pressuring industries to boost production while prioritizing consumer safety. Food contamination poses a significant threat to the manufacturing sector. To address this, experts from various fields have developed protocols, documentation, and procedures to meticulously monitor contamination throughout the food production cycle. These standardized protocols serve two key purposes: preventing cross-contamination and protecting consumers. By rigorously implementing these protocols, food manufacturers can mitigate contamination risks, maintain high quality standards, and safeguard consumer well-being. Regulatory agencies like ANVISA and the FDA play a crucial role in ensuring food safety. By adhering to established standards and undergoing regulatory scrutiny, manufacturers demonstrate their commitment to quality and safety, building consumer trust and mitigating health risks. A critical aspect of food safety is microbiological control. The Clean-In-Place (CIP) protocol, utilizing sanitizing

agents like hypochlorite, peracetic acid, and sodium hydroxide, helps control microbial growth [1–4]. However, the use of chemical solutions in food production raises concerns about environmental impacts and potential health risks. To address these issues, the industry is exploring innovative approaches to minimize chemical use while maintaining high sanitation standards. This includes the development of new technologies and formulations. This study aims to introduce a novel technique that can enhance the manufacturing process while ensuring rigorous contamination control. This approach will be evaluated based on factors such as cost and operational efficiency.

2. Materials and Methods

The procedure used in this study was divided into two main stages: the construction of the pilot reactor home made (IFSC-USP, São Carlos, Brazil) and the evaluation of the effectiveness of the equipment to inactivate microorganisms (ATCC 25922).

2.1. Prototype of Circulation

In this pilot study, conducted on a laboratory scale, a continuous-flow system was utilized to decontaminate water using a UV reactor constructed specially for this purpose. The following basic characteristics are present in Table 1.

Table 1. Characteristics of the system flow.

Characteristic	Parameters
UV-C lamp	4 W
Velocity of the pump	8 L/min
The volume of water used	800 mL
Mass of contamination of broccoli	5 g/each

The flow was contained within a closed-loop system, which consists of a tank (1), where contaminated broccoli was inserted. In this tank, leaching takes place, removing the microorganisms through the water flow. The fluid circulation was facilitated by passing through the UV light reactor.

During circulation, the radiation was delivered to the water to inactivate the leaching microorganisms. This occurred for the entire content of the fluid as it passed through several passages within the reactor. The system operates with 10 circulations per minute.

2.2. Reactor Geometry and Total UVC Delivery

For the characterization of the annular reactor's light distribution, an optical fiber connected to the spectrometer (Ocean Optics USB 2000+UV-Vis) was used. The fiber was placed in the region of interest. From the measured intensity, the delivered energy dose was calculated [1].

2.3. Microbiological Method Analysis

To contaminate the vegetable broccoli, pieces of approximately 9.95 g were used and immersed in a solution with 10^8 CFU/mL of *Escherichia Coli*. After this step, the vegetables contaminated with microorganisms were inserted into the circulation system (Figure 1). During the experiment, samples of both the vegetable and water were taken, and serial dilutions plated onto BHI agar plates were applied to analyze the presence of the microorganisms [5–8].

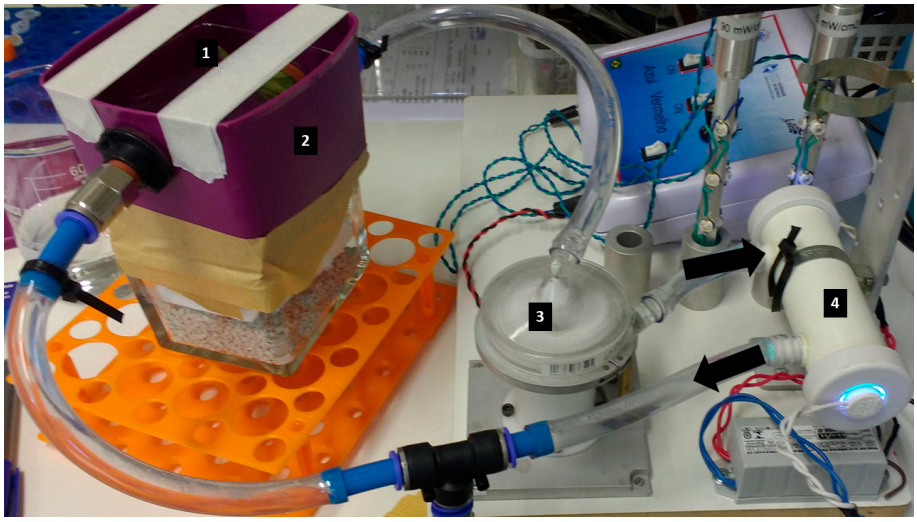

Figure 1. The circulating system to analyze the decontamination of the broccoli has an annular UV light reactor. (1) Tank to perform lixiviation of the vegetable matter; (2) drainage to replace water; (3) pump to recirculate water; (4) UV-divergent reactor.

The microorganism concentration was determined by counting the colony-forming unit (CFUs) per volume (500 mL). Then, CFU/mL was transformed logarithmically to analyze the results [9]. Were performed the descriptive analysis and understood the distribution of the data. Subsequently, ANOVA and Tukey tests were applied to define the statistical relationship between the results. The software used for this analysis was Origin 2022. The significance level was 95% with $\alpha = 0.05$.

2.4. Model of Light Delivery and Photoinactivation

For this experiment, the flow was characterized as non-turbulent while in contact with the UVC radiation.

In this flow regime, well-behaved fluid movement is expected without the formation of bubbles and micro-bubbles, which could scatter the UVC radiation [10]. Therefore, it was ensured that all the UVC light interacts effectively with the fluid content in Figure 2.

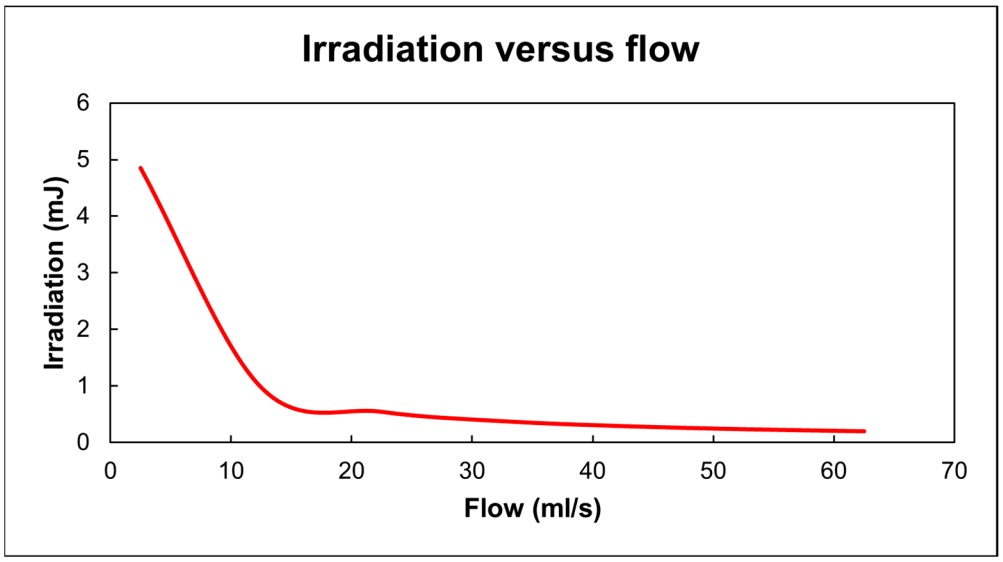

Figure 2. The estimated UVC irradiation profile delivery in the water circulating into the device versus the variation in the flow.

The basic concepts for evaluating the flow profile and the mathematical influence on radiation delivery in the water during the experimental process have been described up to this point [11,12].

In evaluating the flow profile, factors such as flow rate, velocity, and turbulence within the closed-loop system are essential for understanding how the water circulates through the UV reactor. The flow profile influences the distribution of radiation throughout the fluid, impacting the effectiveness of microbial inactivation.

Mathematical modeling plays a crucial role in quantifying radiation delivery within the water. By employing mathematical equations and simulations, the spatial distribution and intensity of UV radiation within the reactor can be predicted. This allows for the optimization of the reactor design and operating parameters to ensure adequate exposure of the water to UV radiation for effective microbial disinfection.

The first part of the study explains the equation of the velocity profile, which describes how the velocity of the fluid varies across different points within the closed-loop system. Understanding the velocity profile is crucial for assessing the flow dynamics and the distribution of the fluid as it passes through the UV reactor.

Therefore, it the general equation of the Lambert–Beer law for light distribution in the reactor present in this research will be described as [13,14]:

$$I(r) = I_0 \frac{R_0}{r} e^{-\alpha(r - R_1)} \tag{1}$$

where I is the UVC radiation intensity traveling into the reactor at distance r, the boundary condition $I = I_0$ is the initial intensity radiation at $r = R_0$ (initial radial distance), and α is the absorption coefficient.

To determine the energy delivery to fluid D, it is necessary to use the relationship of threshold energy in the solution that is described by [3,15]:

$$D = I(r) \times t = \frac{I(r) \times L}{v(r)} \tag{2}$$

where t is the time, L is the length of the radiation section, and v is the fluid velocity.

The principal influence on the kinetic equations is the order of the reactions being considered. In this context, it approaches the microorganism results and describes the below results [16,17]:

$$\ln\left(\frac{[MO_i]}{[MO_o]}\right) = -kD \rightarrow \frac{[MO_i]}{[MO_0]} = e^{-kD} \rightarrow \therefore [MO_i] = [MO_0]e^{-kD} \tag{3}$$

where $[MO]_0$ and $[MO]_t$ are variations in the microorganism concentration, which varies from the initial time to the final, respectively, and k is an inactivation constant.

By starting with the assumption of a first-order reaction and describing these fundamental concepts, it is possible to begin to analyze the kinetics of the reaction and determine how the concentration of reactants or products changes over time. This provides a basis for the mathematical modeling and analysis of the system's behavior.

3. Results and Discussions

The initial emission data quantified from the UV radiation source yielded a value of 2.38 mW/cm^2, which represents the nominal irradiation within the annular reactor configuration. This information is crucial for understanding the intensity of the UV radiation delivered to the water within the reactor [1,4,18].

Additionally, the total volume of water employed in the experiment was measured to be 800 mL. Meanwhile, the internal capacity of the reactor itself was determined to be

147 mL. These outcomes are significant as they provide key parameters for the delineation of the flow profile within the reactor system.

By knowing the volume of water used and the internal capacity of the reactor, parameters such as residence time and flow rate can be calculated. These parameters are essential for understanding how the water flows through the reactor, how long it remains within the reactor, and how effectively it is exposed to UV radiation for microbial inactivation.

To assess the capacity of the system to deactivate microorganisms effectively, it is essential to establish the requisite nominal UV dose for distinct types of microorganisms. The UV dose requirement varies depending on the type of microorganism being targeted. The nominal UV dose requirements for different microorganism types are described according to Table 2.

Table 2. The microorganism species types and dose of UVC irradiation threshold necessary to inactivate the biological system.

Microorganism	Dose (mJ/cm^2)
E. coli 0157:H7 CCUG	3.5
S. aureus ATCC 6538	5.6
Other spore types	14.2
M. radiodurans ATCC13939	198.6

The capacity to illustrate the variance in nominal dosage administered to diverse microorganisms is indeed feasible. This involves understanding the different UV dose requirements as outlined in Table 2. By comparing these requirements, it is possible to visualize the range of UV doses needed to effectively deactivate various microorganisms. Furthermore, an essential requirement involves approximating the direct delivery of irradiation within the flow and evaluating the reactor's ability to render microorganisms inactive [1,4,18]. This evaluation is crucial for determining the effectiveness of the UV disinfection process. Figure 3 depicts the distribution of UV irradiation within the reactor and how it interacts with the flowing water.

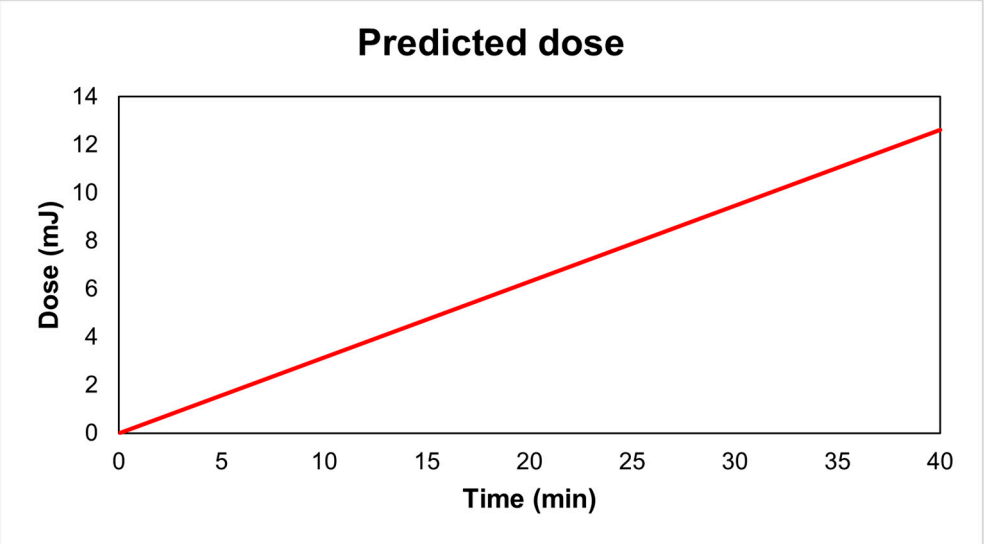

Figure 3. The predicted dose in flow circulating at 62.5 mL/s. The dose delivery, once circulation is complete is 0.194 mJ, and the maximum analysis lasts 40 min. This prediction is estimated in order to determine the dose in the device.

Given that the flow rate employed in this study is approximately 62.5 mL/s, and each data point receives a quantified energy of 0.194 mJ, the comprehensive fluence (total energy delivered) is derived through the incorporation of residence time.

Residence time represents the interval during which the fluid undergoes irradiation across the experimental arrangement, essentially the throughput within the reactor. To accomplish this, it is essential to delineate the control volume, characterized as an infinitesimal unit, and apply Equation (2).

Equation (2) represents an equation relating the fluence (total energy delivered) to the residence time and other pertinent parameters. By applying this equation, the comprehensive fluence received by the fluid as it flows through the reactor can be calculated. This comprehensive fluence accounts for the total energy delivered to the fluid and is crucial for assessing the effectiveness of the UV disinfection process in terms of deactivating microorganisms.

As a result, leveraging these data allowed for the estimation of the complete volume residing within the reactor at 147 mL, while the overall quantity of water employed amounted to 800 mL. The duration necessary for the entirety of the volume to traverse the system at least once is 70 mL. These latter figures establish the correlative framework between dose and temporal variation, a dynamic that found application in the experimental depiction presented in Figure 4.

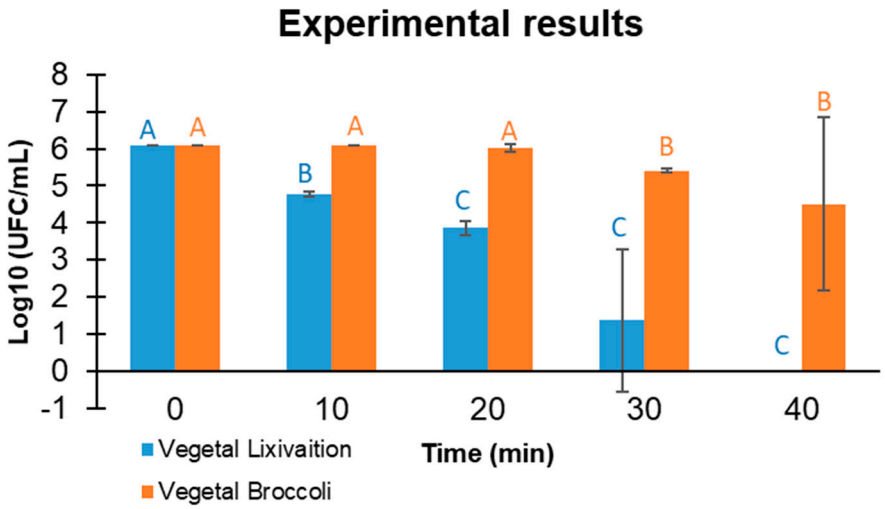

Figure 4. To visualize the results, the contamination of vegetables (blue bars) and water (orange bars) was compared in CFU/mL. Additionally, the residual survival was measured and statistically compared to the results at time points 0, 10, 20, 30, and 40 min, and can be categorized into three statistically differents groups: 0 min (A), 10 min (B), and 20, 30, and 40 min (C).

In the vegetal broccoli, microbiological inactivation occurred, and when the Tukey test was applied, it indicated that there were two groups with a statistically significant difference, surpassing the value of $\alpha = 0.05$. Specifically, at time 0–20 min (Group A), the values were 1, 0.56, and 0.56, respectively. At this point, it is considered that there is no difference between the values within Group A. Conversely, at times 30 and 40 min (Group B), there is no difference observed within the group itself; however, there is a significant difference when this group is compared to Group A. The table below shows the complete data (Table 3).

Table 3. Results of the Tukey test with values when compared to samples.

Samples Compared	α	Vegetal Broccoli	Vegetal Lixiviation
10→0	0.05	1	0
20→0		0.565	0
20→10		0.56	6.07×10^{-6}
30→0		1.65×10^{-5}	0
30→10		1.65×10^{-5}	2.98×10^{-6}
30→20		7.68×10^{-5}	0.91
40→0		2.22×10^{-6}	0
40→10		2.22×10^{-6}	2.01×10^{-6}
40→20		8.04×10^{-6}	0.47
40→30		0.21	0.91

Having elucidated the dose dynamics and garnered endorsement for these mathematical outcomes, the research transitioned into the experimental phase, with a concentrated emphasis on microbiological considerations. The initial stride in this segment encompassed substantiating the viability of leaching vegetable constituents within this configured apparatus, operating under the stipulated physical property parameters.

To achieve this objective, the controlled introduction of clean water was administered into the system, coupled with the incorporation of broccoli, known to harbor E. coli within its foliage. The initial concentration of the microorganism was set at 10^6 CFU/mL. The ensuing outcomes are graphically depicted in Figure 5.

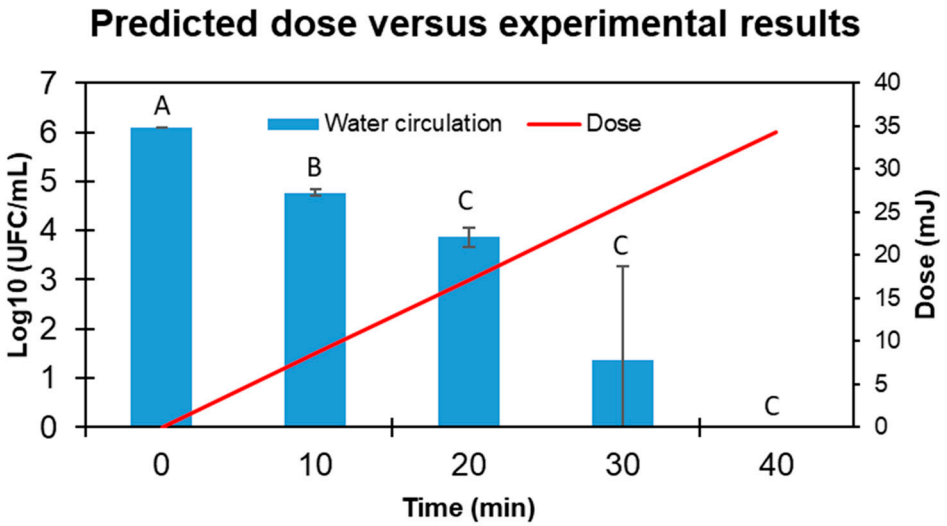

Figure 5. The experimental microbiological results were compared to predict dose delivery in the system. The left axis is CFU/mL (experimental results), and the right axis describes the estimated dose delivery to the circulation fluid at 62.5 mL/s and with 800 mL being the total volume contained in the system. Soon, the experimental inactivation in the system versus the calculated dose delivery evolved over time. The water circulation has three groups with statistical differences between them, 0 min (A), 10 min (B), and 20, 30, 40 min, with (C) being the last group.

The contaminated water was recirculated and irradiated to achieve the above shape. Guided by theoretical insights, it was ascertained that a duration of 35 to 40 min of fluid recirculation was imperative for the deactivation of microorganisms within the solution. Following this procedural phase, the assessed outcomes about both the vegetable and the water

within the system were graphically represented. This graphical representation juxtaposes the initial concentration of microorganisms against the temporal dimension (Figure 5).

The final phase entailed the determination of the kinetics law and the establishment of the mathematical order and dynamics governing microorganism inactivation. Initially, it was inferred that the process adheres to first-order kinetics. Subsequently, a comparison was conducted between the experimental concentration rate over time and the mathematical kinetics law, with the outcomes succinctly presented in Figure 6.

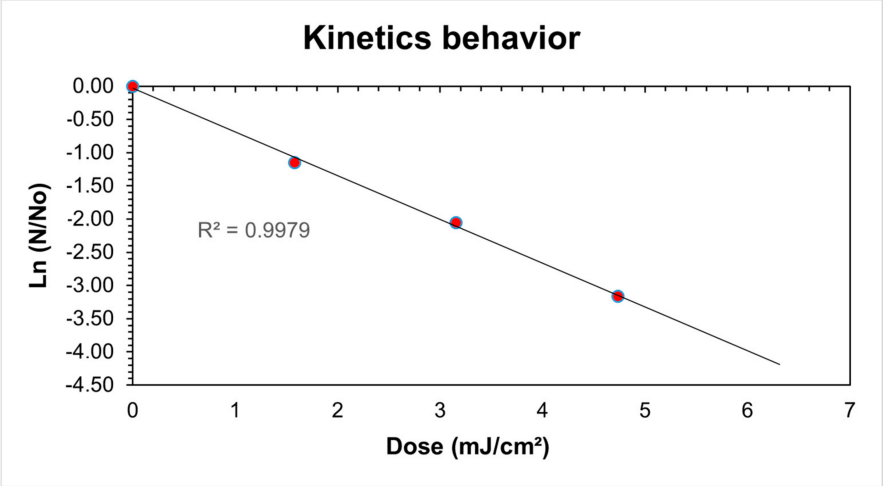

Figure 6. To determine the kinetics level law, the first step is the ratio of the initial and final concentrations versus the irradiation delivery, and consequently, one-line graphic results were plotted and the R^2 calculated. If the number of approximately 1 is first-order, the test is in the order levels. In this case, the other kinetics are first-level.

The behavior of the inactivation rate of the microorganism by dose is described in the kinetics laws of the first order, thus the experimental results will have a shape close to the curve, for it plots the estimated curve versus the experimental to increase the dose irradiation level (Figure 7) [1,4,18].

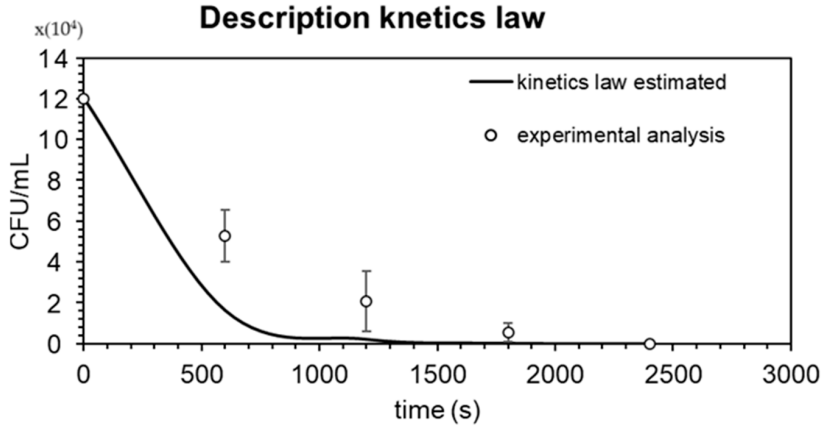

Figure 7. To validate the theoretical aspects of the kinetics law estimate, is necessary to develop the theoretical curve, and also insert the experimental results; this point is needed to analyze the overlap between the experimental and theoretical results obtained. In this case, the experiment showed a smaller level of inactivation than was theoretically estimated; this occurred because the microorganisms can regenerate the damage inflicted, and the kinetics equations did not account for this.

The results above include a comparison of the experimental results obtained and those estimated by the kinetics law. It can be observed that the shapes do not overlap at the initial stage of irradiation.

This observation stems from the protective mechanisms present in microorganisms. When exposed to irradiation doses below the threshold necessary to break the bonds and/or cause DNA/RNA protein deconfiguration, microorganisms generate photopolymers.

In the kinetics law, this aspect of the equation formulation was not considered, resulting in a disparity between the theoretical and experimental results.

The imperative requirement for advancing novel technologies to bolster productivity, curtail expenditures, and concurrently alleviate environmental harm is progressively escalating.

The impact of ultraviolet radiation on DNA has been extensively documented in the scientific literature over a prolonged period. Depending on the wavelength of incident UV radiation, two distinct categories of damage manifest within the DNA structure. In the UVA range (320–400 nm), the predominant influence is that of indirect effects, owing to the lack of light absorption by nucleic acids within this domain. Nevertheless, this radiation can potentially engender the production of reactive oxygen species (ROS) [19,20].

ROS emerge from the oxidative processes involving molecular oxygen within mitochondria and other molecules. This oxidative activity leads to the generation of superoxide (O_2^-), which subsequently has the potential to evolve into hydrogen peroxide (H_2O_2) and molecular singlet oxygen (1 O_2). These ROS can oxidize nitrogenous bases, incite strand fractures, and even precipitate DNA protein cross-linking [21,22].

Within the scientific literature, the documented minimal nominal dosage needed for the deactivation of *E. coli* ATCC 8739 is approximately 8.1 mJ/cm^2, whereas for *E. coli* 0157:H7 CCGU 29193, it rests around 3.5 mJ/cm^2. In the scope of the present study, the circulation system, operating at a flow rate of 62.5 mL/s, demonstrates the potential to administer a dosage of up to 12 mJ. This highlights that the system can provide fourfold the requisite energy for microorganism inactivation.

These findings signify a 99% and 99.999% diminishment of microorganisms, respectively, compared to their initial concentrations at time zero. The ensuing inquiries pertain to the correlation between time and microorganism inactivation, alongside the reactor's efficacy in curtailing biological activity.

The employed technique reduced 90% of microorganisms within 20 min, 99.9% within 30 min, and 99.999% within 40 min. This condition underscores the efficacy of microorganism inactivation and facilitates the formulation of a theoretical estimation, approximating that around 35 min are requisite for specific outcomes—a projection remarkably close to actuality.

Nevertheless, in the context of irradiation of the vegetable, the outcome deviates from that observed in the case of water. This divergence can be attributed to the presence of a vortex induced during the leaching procedure of the broccoli within the tank. It becomes imperative to enhance mixing efficiency in this scenario. This objective could be accomplished by augmenting the separation between low-pressure and high-pressure regions, attainable through the elevation of the flow rate or the introduction of a barrier during the re-circulation phase [22].

Microbiological inactivation by UVC radiation occurs initially through the absorption of radiation by DNA. In this process, two main excited states, $1\pi\pi^*$ or $1\ n\pi^*$, are formed due to the absorption of photons by pyrimidine molecules present in the nitrogenous bases [22].

Consequently, the process triggers the formation of pyrimidine dimers, resulting in the generation of three distinct photoproducts: 6-4 photoproducts (6-4 PPs), alongside Dewar dimers. Within this spectrum, it is the cyclobutene pyrimidine dimers (CPDs) and 6-4 photoproducts (6-4 PPs) that inflict the most substantial harm to DNA, accounting for 70–80% and 20–30%, respectively.

The dosage administered to the microorganism facilitates the generation of these photoproducts, subsequently culminating in the inactivation of the bacteria. Consequently,

a reduced dosage administered to the microorganism possesses the inherent potential for regeneration, thereby facilitating the recovery of the microorganism.

4. Conclusions

In summary, the findings presented here highlight the viable prospect of leaching alongside the simultaneous attainment of efficient microorganism inactivation within the vegetable. Notably, the experimental investigation centered on broccoli, acknowledged for its intricate leaf structure that imparts intricacies to the leaching process.

What is particularly intriguing is the close concordance observed between the mathematical predictions and the empirical outcomes. This convergence robustly affirms the prospective success of microorganism inactivation within the circulatory framework, thereby enhancing the reliability of our conclusions.

As a result, this study makes a significant stride in the domain of food engineering, propelling the field of contamination control techniques to new heights. Importantly, it unequivocally underscores the possibility of circumventing reliance on auxiliary chemicals for microorganism inactivation. Indeed, it underscores the paramount importance of implementing prudent measures to bolster production while concurrently mitigating environmental impact.

5. Challenges

UV technology offers a promising approach to water decontamination by inactivating microorganisms through DNA damage. However, several challenges hinder its widespread and effective implementation:

1. Turbidity and suspended solids: UV light is absorbed by particles in water, reducing its penetration depth and disinfection efficacy, and high turbidity can significantly diminish the effectiveness of irradiation.
2. UV lamp degradation: UV lamps gradually lose intensity over time, requiring regular replacement or maintenance to ensure effective disinfection.
3. Limited penetration depth: The irradiation by UV has limited penetration into water, especially at certain wavelengths. This can restrict the effectiveness of UV treatment in deeper or more turbid water bodies.
4. Potential for byproduct formation: While generally considered safe, irradiation by UV light can potentially lead to the formation of disinfection byproducts (DBPs) in certain water conditions. These byproducts may have adverse health effects.
5. Limited effectiveness against certain pathogens: Some pathogens, such as Cryptosporidium and Giardia, are more resistant to UV inactivation than others. This necessitates higher UV irradiation or combined treatment methods to ensure complete removal.
6. Potential for shadowing: In some UV reactor designs, water flow can create shadows, shielding microorganisms from light and reducing disinfection efficacy.

6. Patents

BR1020180002082—“Dispositivo Fotônico Descontaminador de Fluídos Industriais”, concession: 2019.

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