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The antimicrobial effect of ultra–violet on pathogenic microorganisms in an aquatic medium

N.Rhaiem[1]*, M.Sobh[1], A.Qebibo[1], R.Amiyare[1], M.Ouhssine[1]

Laboratory of Biotechnology, Environment and Quality, Department of Biology, Faculty of Science, Ibn Tofaïl University, BP 133 Kenitra 14000, (MOROCCO)

E-mail: rnourd@gmail.com

ABSTRACT

The UV radiations have become an effective way for the sake of solving all problems related to environmental contamination. An example of that is their use in the field of water treatment. The effectiveness of UV in inhibiting the growth of pathogens can be seen in several case studies. This inspiration led us to choose the UV and see their degree of implication on our pathogenic microorganisms (of our micro- library). To materialize this, we propose monitoring the effect of the time and distance of exposure factors on the control microbial growth. In addition, it is good for us to determine the minimum inhibitory time (MIT) and the minimum exposure distance (MED) on each bacterial type.

Tests are carried on the following microorganisms: Klebsiella pneumoniae, Staphylococcus aureus, Pseudomonas aeruginosae, Escherichia coli, Enterobacter cloacae and Proteus mirabilis. The cultures were irradiated with UV. The selected distances are 3 and 13 cm and the exposure time was 10 min, 20min, 30 min, 40min and 50min. All treatments are carried out in a dark chamber. Further, the control group is prepared in the same conditions. The extermination of most microorganisms has been viewed due to the combined effect of time and the exposure distance. The time of 40min and a distance of 3 cm was the best combination to achieve the total cessation of growth of the bacteria studied.

KEYWORDS

UV;
Exposure time;
Exposure distance;
Pathogenic microorganisms.

INTRODUCTION

In a consumer market where food security is compulsory, manufacturers should take into account the Moroccan consumers expectations related to the standards of quality, non- microbial growth, and moderate use of colors. Their frequent outcries have been repeated against the use of chemical additives. Disinfection by UV radiation can be alternative antimicrobial additives.

UV radiation is used as a mean of conservation and sterilization[1,2]. Their effectiveness is published in several researches[3,4]. Areas of use of UV are known such as air, pool water, wastewater, food products[3] and even the medical field.

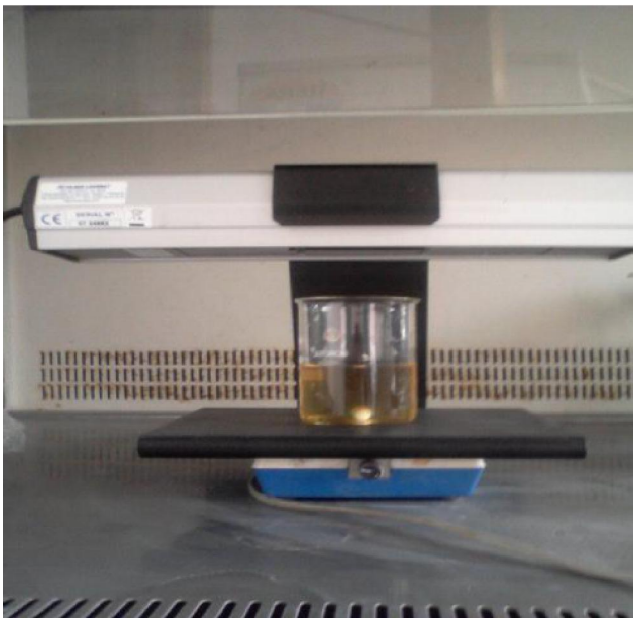
In our study, we will use (UV) against certain microorganisms that harm food and hygiene interest. Fur-

ther, Microorganisms continue to pose serious problems in food industry (as in production chains)[5,6]. It is the same in hospitals (where corridors and benches)[7]. Although, these areas are sterile, they become vulnerable to any pathogen accident. This urges us to expand our research and be prepared to complete total aseptic and follow sterilization measures and respect the hygiene.

We used the UV lamp of wavelength of 254 nm. The choice of this length is based on the fact of its denaturing of the DNA[8], its ability to modify the structure of membranes and damage vital cellular components and the extermination of pathogenic bacteria. The objective of this work is to reach the exposure time and distance to exterminate the pathogenic bacteria.

MATERIALS AND METHODS

The UV device used in the laboratory is provided with a UV lamp with two different wavelengths (254 nm and 324 nm). (See the dispositive below).



In order to stop the proliferation of pathogenic bacteria, a sample exposed to two wavelengths 254 nm and 324 nm at first. Then, it was inoculated in a Petri dish and the agar medium was added to it. The growth is evaluated by the counting method on solid medium after incubation[9].

The objective is to distinguish between the sensitive bacteria and the resistant ones to UV radiation. The low inhibitory average was expressed by the time and the distance of exposure.

Microorganisms and culture conditions: Microorganisms

Pathogenic microorganisms studied are*: Escherichia coli, Pseudomonas aeruginosae, Klebsiella pneumoniae, Proteus mirabilis,, Staphylococcus aureus and Enterobacter cloacae.*

Culture conditions: Processing mode (In Biofilm)

The used cultivation method is planctonic; the samples were prepared in the same culture conditions. A preculture of 24 hours in nutrient broth was made. 1ml of 10^{-6} dilution is inoculated into the Petri dish and the nutrient agar was added to it. After 24 hours of incubation, one to two colonies are isolated, depending on size, were taken and were inoculated in a nutrient broth, after 18 hours to 24 hours, 15 ml of the preculture was transferred to a final volume of 150 ml of the nutrient medium and has already sterilized and has been put under UV lamp at 254 nm. The medium is subjected to continuous agitation. 1ml of medium was inoculated in a Petri dish every 10min. The time of 0 min corresponds to the control groups. The dilution was made in a physiologic liquid until 10^{-6}. The microbial load is adjusted to that of the standard McFarland and the final concentration is set at 10^6 ufc/ml. After cooling, the plate was placed in an incubator with adjustable temperature for 24 hours. The effect of UV is evaluated by counting viable colonies on solid medium[10].

RESULTATS AND DISCUSSIONS

All bacteria that are gram positive or gram negative are exposed to both existing wavelengths of 254 nm and 324 nm in the lamp, but we noticed that only the wavelength of 254 nm is the effective length than 324 nm which has weak effect on stopping the growth of pathogens that is why the first one was chosen to continue the coming tests.

Pictures 1 and 2 show the comparison of the exposure of *Escherichia Coli* to two different wavelengths for 20 min result.

The two plates are seeded with the same way by *Escherichia Coli*. Correspondingly, both plates seeded

by the samples exposed to the two wavelengths (254 nm and 324nm). The result after 24 hours of incubation showed an obvious growth of bacteria in the case of the wavelength of 324 nm (Figure 1). It is not the same result with the exposition at 254nm (Figure 2).

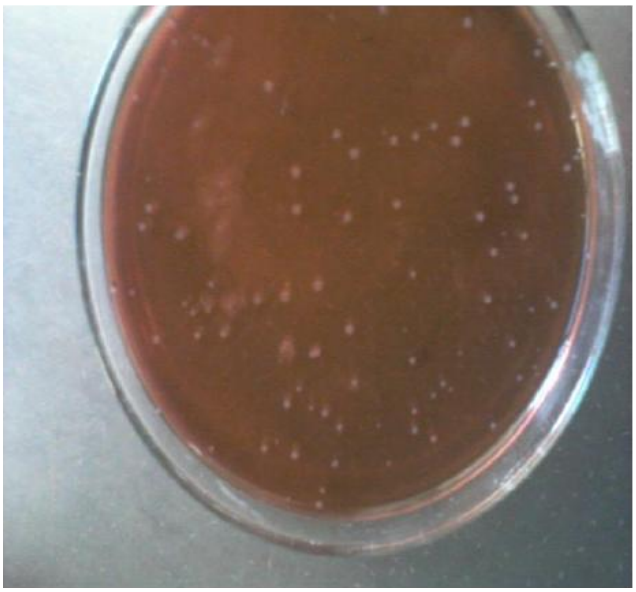


Figure 1 : E.C exposed to UV at 324 nm; E.C: *Escherichia Coli*

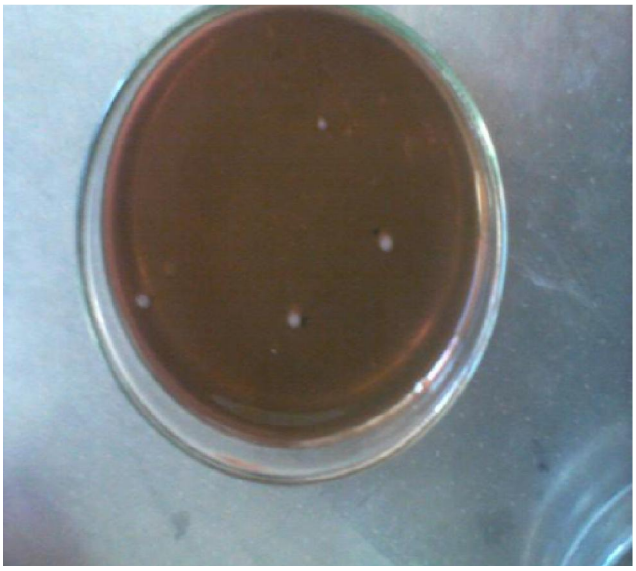


Figure 2 : E.C exposed to UV at 254 nm; E.C: *Escherichia Coli*

The perturbing effect of the vital functions that led to the extermination of *Escherichia Coli* was noticed with the radiations of 254 nm. Hence, the result guided us to choose the latter for the physical treatment against the other bacteria studied.

The results obtained after the exposure of the bacteria are grouped in tabular form for each specy:

Proteus mirabilis is a very mobile gram-negative bacterium, is very sensitive to UV radiations. We have noticed the existence of some microorganisms in 30 min. The total inhibition of the growth was not observed just in 40 min at a distance of 3 cm. Thus, the needed time for a total eradication of the microorganisms is 50 min for both distances (TABLE 2).

Staphylococcus aureus is a coagulase-positive bacterium, but according to literature; it has been shown a high sensitivity to UV[11], In this case, 30 min of its exposure was sufficient to inhibit multiplication of the bacteria at a distance of 3 cm. In the opposite, the total inhibition of this bacterium has not been obtained at a distance of 13 cm only in 50 min (TABLE 3).

Escherichia Coli is a microorganism which is often associated with the hygienic quality of washing water. It is considered as an indicator of fecal contamination. The absence of the microorganisms on the solid medium has been obtained only in 30 min of exposure at 254 nm and for the distance of 3 cm. Hence, the radiations have not completely exterminated the bacteria at the distance of 13 cm (TABLE 4).

Enterobacter cloacae is a gram negative bacterium, facultative anaerobic, oxidase negative and catalase positive. In a Petri dish, we have observed the existence of the microorganisms in 30 min. Their total absence has not been achieved only in 40 min of the exposure to radiations of 254 nm at the distance of 3cm. For both distances, 50 min is the perfect time to eliminate the bacteria totally (TABLE 5).

Pseudomonas aeruginosae is a gram- negative and multiresistant bacterium[12]. The UV radiation inhibits the growth of the microorganisms for the exposure of 30 min and at a distance of 3 cm. At a distance of 13 cm, the Petri dish contains the same microorganisms for the same time. The absence of the bacteria has not

TABLE 1 : The nature of microorganisms by gram type

Microorganismes	gram +	Gram-
Klebsiella pneumoniae		+
Staphylocoque aureus	+	
Pseudomonas aeruginosa		+
Escherichia coli		+
Enterobacter cloacae		+
Proteus mirabilis		+

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TABLE 2 : The effect of U.V on *Proteus mirabilis*

Exposure Time (min)	0	10	20	30	40	50
3cm, cfu / ml	178.10^6	33.10^6	21.10^6	12.10^6	0	0
13cm, cfu / ml	178.10^6	64.10^6	48.10^6	36.10^6	4.10^6	0

TABLE 3 : The effect of U.V on *Staphylococcus aureus*

Exposure time (min)	0	10	20	30	40	50
3cm, cfu / ml	160.10^6	24.10^6	10.10^6	0	0	0
13cm, cfu / ml	160.10^6	31.10^6	21.10^6	9.10^6	2.10^6	0

TABLE 4 : The effect of U.V on *Escherichia Coli*

Exposure time (min)	0	10	20	30	40	50
3cm, cfu / ml	138.10^6	30.10^6	4.10^6	0	0	0
13cm, cfu / ml	138.10^6	88.10^6	27.10^6	9.10^6	3.10^6	0

TABLE 5 : The effect of U.V *on Enterobacter cloacae*

Exposure time (min)	0	10	20	30	40	50
3cm, cfu / ml	162.10^6	47.10^6	25.10^6	10.10^6	0	0
13cm, cfu / ml	162.10^6	60.10^6	32.10^6	21.10^6	9.10^6	0

TABLE 6: The effect of U.V on *Pseudomonas aeruginosae*

Exposure time (min)	0	10	20	30	40	50
3cm, cfu / ml	280.10^6	198.10^6	46.10^6	0	0	0
13cm, cfu / ml	280.10^6	213.10^6	24.10^6	5.10^6	2.10^6	0

TABLE 7 : The effect of U.V *on Klebsiella pneumoniae*

Exposure time (min)	0	10	20	30	40	50
3cm, cfu / ml	203.10^6	103.10^6	50.10^6	5.10^6	1.10^6	0
13cm, cfu / ml	203.10^6	156.10^6	80.10^6	25.10^6	4.10^6	0

been obtained only in 50 min (TABLE 6).

The examination of the effect of UV on *Klebsiella pneumoniae* was studied. As a reminder, *Klebsiella* is a gram negative bacterium, involved in nosocomial pneumonia and it is sensitive to UV radiations. The total destruction of such a pathogenic microorganism in the aquatic medium (100 % of inhibition) was observed in 50 min and at a distance of 3 cm. The distance of 13 cm was not as effective in removing all bacteria (TABLE 7).

At the end of what has preceded, we have derived the following information: UV radiation has the potential to inactivate pathogenic microorganisms[13], particularly for the wavelength 254 nm, for a contact time of 50 min, and for a distance of 3 and 13cm. Then, we have noticed a significant difference in the achieved results between both chosen distances for the wavelength of 254 nm.

CONCLUSION

The tests in this work are carried out in order to verify the effect of UV radiation on the growth of some pathogenic microorganisms.

All experiments were done in three attempts and the incubation of bacteria was at 37 ° C for 24 hours. The antimicrobial effect of the UV lamp is determined by following the parameters of the time and distance of exposure to UV toward the pathogenic microorganism tested.

The result was quit the same for all the bacteria mentioned above. The exposure of microorganisms studied to UV light for five times and two different distances, led to a significant reduction in the number of viable bacteria.

The bactericidal action was important for the ex-

posure of 40 min. Similarly, UV radiations kill pathogenic bacteria at a distance of 3cm. Furthermore, the more we move away from the UV source the more likely the killing of bacteria is reduced. For a distance of 13 cm, we obtained a reduction in the number of bacteria but not a total eradication.

The bactericidal effect of UV is confirmed through experimentation maintained in this work. The distance of 3 cm and the time of 50 min are sufficient to kill all pathogens in the aquatic medium[9]. Thus, we have confirmed the hypothesis raised in the introduction.

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