# EFFECT OF DIFFERENT LED WAVELENGTHS ON SURVEILLANCE CURVE OF *ESCHERICHIA COLI* AB1157 AND BW9091 WITH POSSIBLE GENOTOXIC ACTIONS

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**ABSTRACT:** Physiotherapy plays a function in the facilitation of repair process, once it utilized resources as laser and ultrasound therapy, sonic waves, microwaves and the phototherapy for processing the modulating repair. The utilization of the LED (Light Emitting Diode) in the wound healing process is based on its effects antibacterial, anti-inflammatory and cicatrization. The objective of this work is to investigate the effects of the LED of different wavelengths on surveillance of one cellular type proficient in repair DNA damage. It was used *Escherichia coli* (E.coli) strains proficient in repair DNA damage (AB1157) or not proficient (BW9091). Exponentially *E. coli* AB1157 or BW9091 cultures were incubated at liquid rich medium overnight. Aliquots were spread onto Petri dishes containing solidified rich medium and irradiated with LED of different wavelengths, the colonies units were counted after overnight and the survival fraction was calculated. The results show cytotoxic effect of blue LED on *E. coli* AB1157, and of the green and blue LEDs on *E.coli* BW9091. In conclusion, the results of bacterial surveillance showed cytotoxic effect manly for blue light (bacterial effect) and green light (anti-inflammatory effect).

**Keywords:** LED. *Escherichia Coli.* DNA. Surveillance. Healing

## 1 INTRODUCTION

The process of cicatrization has during last year been the motive of researches attention with relation the factors that it could difficulty this process. It is a physiological process, complex and highly organized that initiate with an inflammatory answer, characterized by elevation of the blood flux, capillary permeability and leucocytes migration to the damage region. The factors that difficult the repair are the contaminated field inside what occur the infection, the nutritional deficit state, manly the hypoproteinemia, system diseases associated with diabetes, radio-chemotherapy, chemotherapy and the use of

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immunosuppressive drugs. One of the more efficient properties of our organism defense is the organic capability of solve a wound. Solutions for doing better the quality of patient life that have deficiencies on the tissue repair process are been investigated (LEBLANC et al., 2011; VASHEGHANI et al., 2008).

The physiotherapy has an important function in the facilitation of the repair process, once it utilized resources capable the modulation of this process. Phototherapy, sonic waves and ultrasound are one of the resources utilized. One option of phototherapy used today is the LED (*Light Emitting Diode*) that is a semiconductor diode submitted to electric current that emits light and it is utilized for phototherapy with different wavelengths between 405nm (blue) to 940nm (infrared). The photo-stimulation by this light acts in the cell and on their permeability and on mitochondria stimulation, ATP synthesis, collagen, proteins and elastin. This light also act as causing antimicrobial and anti-inflammatory reactions and because it they are indicated to many inflammatory affections (ABRAMOVITS; ARROZOLAP, 2005).

Laser and LED are too close in the point of view of the emission of light, both produce an spectral band relatively sharp, although LED have an spectra more large. The important difference is about the emergent light of LED that is not collimated. So, LED is a good alternative in relation to slow intensity to promote the wound healing (CORAZZA et al., 2007).

Laser and LED with different doses of energy produce effects on blood vessels of the skin. The phototherapy based on Arndt-Shultz curve is utilized to stimulate the production of endothelial cells and angiogenesis, and the production of fibroblast and collagen (REDDY, 2003; PEREIRA et al., 2002). In another way, some authors justify his utilization by elevation the photo-modulation of wound healing (AL-WATBAN et al., 1999).

The idea of utilize LED on cicatrization process have been based in the works that confirm its effects antibacterial, anti-inflammatory and cicatrization. Abramovits and Arrozolap (2005), show this new therapeutic, of decrease pain, having quickly resulted and that it used very simple equipment. Their action not promotes tissue damage and occurs through of the intercellular directly stimulation, acting especial in the mitochondria, re-organizes the cells, inhibited the action of some cellular groups and stimulating others. There are different biological and physiological effects according with each LED wavelength, the blue (440-490nm) is bacterial, the green (490-565nm) healing and fibroblast stimulator, the Yellow (590-630nm) healing and collagen stimulator and the red (630-780nm) anti-inflammatory.

Free radicals are high reactive molecules species that could be produced by some cellular oxidative mechanism (INFANGER et al., 2006). If this molecules are produced in excess they could induced tissue damage. There are intern mechanisms in the leaving organism constituted by enzyme and other molecules to protect the cells these free radicals (FR) (HSIEH et al., 2005; KINOSHITA et al., 2005; BAO; LOU, 2006; MARCUS et al., 2006). These defense mechanisms need determinate medicinal compounds including vitamins and others nutritional products that could inhibit the production of free radicals (INFANGER et al., 2006; BARBOSA-FILHO et al., 2008).

Free radicals have been related to primary molecules on a lot of ambient conditions, as well as this species are involved in other biologic phenomena, as mutagenesis, apoptosis and elderly (OZBEN, 2007).

Assays with *Escherichia coli (E. coli*) DNA repair mechanisms deficiency suggest that these chemical agents could induce different lesions in the DNA (EL-DEMERDASH et al., 2005; ALMEIDA et al., 2007). Enzymes that are involved in the base excision repair (BER) for neutralize the DNA damage have been studied in *E. coli* cultures, as exonuclease III, endonuclease IV and formamidopyrimidine-DNA-glycosylase, codified by xth, nfo and fpg genes, respectively. The base-related damage produces lesions with mutagenic properties, contributed to possible carcinogenesis in superior organisms (OLINSKI et al., 1998). The exonuclease III protein (xthA) contributes with almost 90% of the endonucleolytic apurinic/ apyrimidinic activity found on *E. coli*. The xthA product is high important on BER to eliminate the DNA oxidative stress (SOUZA et al., 2006).

This study has the objective investigate the effects of different LED wavelengths in the surveillance process of cell type proficient in repair DNA damage, important to the process of tissue wound healing and to demonstrate which type of LED with specific wavelength, if red (630-780nm) or green (490-565nm) or blue (440-490nm) or yellow (590-630nm) is more efficiency in produce photo-stimulation of the repair mechanism in *E. coli* proficient (AB1157) or not (BW9091) in DNA repair mechanism.

## 2 MATERIAL AND METHODS

The *E. coli* AB1157, a wild-type strain, proficient to repair damage in the DNA, and *E. coli* BW9091, a mutant-type strain, not proficient to repair damage in the DNA, were used in this work. From stock (in glycerol 50% v/v) a sample (50µl) of the culture was grown on liquid LB medium (5ml, Lúria and Burrous, 1957) at 37°C overnight on a shaking water bath

(reciprocal water bath shaker, model R76, New Brunswick, USA) up to the stationary growth phase. A sample (200μl) was taken from this culture and further incubated (20ml; liquid LB medium) under the same conditions to exponential growth ($10^8$ cells/ml). The cells were collected by centrifugation, washed twice in 10 ml of saline and suspended again in the same solution until they reached $10^8$ cells/ml. Samples (0.8 ml) of these washed cultures ($10^8$ cells/ml) were treated according the following protocol: (i) 0.2 ml of 0.9% NaCl (saline solution); (ii) 1 min of Red LED and 0.1 ml of saline solution; (iii) 1 min of Blue LED and 0.1 ml of saline solution; (iv) 1 min of Green LED and 0,1 ml of saline solution; (v) 1 min of Yellow LED and 0.1 ml of saline solution; on initial time, after 30 and 60 min. During the assay, at zero, 30 and 60 min, aliquots (100 μl) were diluted with saline and spread onto Petri dishes containing solidified LB medium (1.5% agar). Colonies units formed after overnight incubation at 37 °C was determined. The survival fraction was calculated dividing the number of viable cells obtained per ml in each time of the treatment (N) by the number of viable cells obtained per ml in zero time (N0).

## 3 RESULTS

Figures 1 to 4 show the results of the application of different wavelengths and the surveillance fractions of *E. coli* proficient in DNA repair (AB1157) when compared to surveillance fractions of *E. coli* AB1157 incubated with 0.9% NaCl (control solution; saline).

It showed more surveillance of the *E. coli* AB1157 to the red LED (80%, in 30 min) and green LED (stabilized to 40% in 30 min) than blue LED (10% in 30 min) and yellow LED (20% in 60 min).

In the figures 5 to 8 we showed the results of submission to different LED wavelengths and the surveillance of *E. coli* not proficient in repair of DNA damage (BW9091) when compared with surveillance fraction of *E. coli* BW9091 submitted to saline (control).

It was verified the total reduction of the surveillance of *E. coli* BW9091 to green LED, 10% to red LED when incubated at 30 min with total reduction at 60 min of incubation, same surveillance to the yellow LED at 30 and 60 min of incubation, and 50% at 30 min with reduction to 10% at 60 min when submitted to the blue LED.

## 4 DISCUSSION AND CONCLUSION

The mainly use of the LED as therapeutic treatment is it help on cicatrization by local application of light with specific wavelength, irradiated potency and determinate time for local application. The important is to obtain the desire wavelength and the sufficient potency to reach the more deep or superficial parts of the skin (VINCK et al., 2005). The use of the LED has a clear advantage it offers different color lights to tissue area (NICOLAU, 2001; VINCK et al., 2005). The application of LED in human tissue is without pain and it is not a traumatic treatment, having a good answer, because it realize natural stimulation on cellular level (VINCK et al., 2005).

Assays with E. *coli* deficient in DNA repair mechanisms showed that chemical agents produced by photo stimulation could induce different damage on DNA (El-Demerdash et al., 2005; Almeida et al., 2007). Enzymes involved the base excision repair (BER) efficient to neutralize the DNA damages have been studied in *E. coli* strains, as well as exonuclease III, endonuclease IV e formamidopyrimidine-ADN-glycosylase, codified by the genes xthA, nfo and fpg, respectively. The damage related to the nucleotide base produce damage with mutagenic properties, contributed for possible carcinogenesis in superior organisms (Olinski et al., 1998). The protein exonuclease III (xthA) contribute with about 90% of endonucleolytic apurinic/ apyrimidinic activity founded in *E. coli.* The product of the gene xthA is much important no BER to eliminate the DNA oxidative stress (SOUZA et al., 2006).

The results obtained revel cytotoxic effect of LEDs in *E. coli* proficient on DNA repair (AB1157), in the tested wavelengths (Figures 1 to 4). The *E. coli* AB1157 was not capable of survive to blue LED (10% in 30 min of exposition) (Figure 4). This is easy explained by the fact that this wavelength has bacterial action. *E. coli* AB1157 survive when it is submitted to red LED (Figure 1), what can be explained by the red LED anti-inflammatory effect (ABRAMOVITS; ARROZOLAP, 2005).

The results also shown that the different wavelengths decreased the surveillance of *E. coli* BW9091 (Figures 5 to 8). The total reduction of the surveillance of *E. coli* BW9091 to the green LED with 30 min of incubation, can be due to it action in fibroblasts on wound healing process where it act as stimulator agent (ABRAMOVITS; ARROZOLAP, 2005). The more interesting result and deserves better investigation was the surveillance in 50% at 30 min of incubation for the blue LED, maybe explained by the action of this wavelength on DNA using direct mechanisms (absorption and photo-products) or indirect (free radicals).

This results could be explained by: (i) LEDs antioxidants properties; (ii) free radicals production by LEDs; (iii) direct absorption of LEDs by DNA creating photo-products on DNA that it modified the production of necessary proteins to the cellular functions.

In conclusion, the results obtained with the use of red (630-780nm), green (490-565nm), yellow (590-630) and blue (440-490nm) lights on surveillance of bacteria strains proficient or not in DNA repair mechanisms showed a cytotoxic effect caused by blue and green lights that it can be better explained in future investigations with the association of other wavelengths used in phototherapy.

## REFERENCES

ABRAMOVITS, W; ARRAZOLA P; GUPTA, A. K. Light-Emitting Diode-Based Therapy. **Derm. Clin.,** p. 38-40, 2005.

ALMEIDA, M. C. Protective effect of an aqueous extract of Harpagophytum procumbens upon Escherichia coli strains submitted to the lethal action of stannous chloride. **Cell Mol. Biol.,** v. 53, p. 923-927, 2007.

AL-WATBAN, F. A. H.; ZHANG, X. Y. The acceleration of wound healing is not attributed to laser skin transmission. **Laser,** v. 11, n. 1, p. 6-10, 1999.

BAO, M.; LOU, Y. Flavonoids from seabuckthorn protect endothelial cells (EA.hy926) from oxidized low-density lipoprotein induced injuries via regulation of LOX-1 and eNOS expression. **J. Cardiovasc. Pharmacol.,** 48 834-841, 2006.

BARBOSA-FILHO, J. M. Sources of alpha-, beta-, gamma-, delta- and epsilon-carotenes: A twentieth century review. **Rev. Bras. Farmacogn.,** v. 18, p. 135-154, 2008.

CORAZZA, A. V. et al. Photobiomodulation on the angiogenesis of skin wounds in rats using different light sources. **Photomed. Laser Surg.,** v. 25, n. 2, p. 102-106, 2007.

EL-DEMERASH, F. M.; YOUSEF, M. I.; ZOHEIR, M. A. Stannous chloride induces alterations in enzyme activities, lipid peroxidation and histopathology in male rabbit: antioxidant role of vitamin C. **Food Chem. Toxicol.,** v. 43, n. 12, p. 1743-52, 2005.

HSIEH, C. L. et al. Gastrodia elata BL mediates the suppression of nNOS and microglia activation to protect against neuronal damage in kainic acid-treated rats. **Am. J. Chin. Med.,** v. 33, p. 599-611, 2005.

INFANGER, D. W.; SHARMA, R. V.; DAVISSON, R. L. NADPH oxidases of the brain: distribution, regulation, and function. **Antioxid. Redox Signal.,** v. 8, n. 9-10, 1583-96, 2006.

KINOSHITA, N. Protection by antioxidants of copper-induced decline of proliferation and SOD activity. **Anticancer Res.,** v. 25, p. 283-289, 2005.

LEBLANC, E. Screening for and Management of Obesity and Overweight in Adults. Evidence Report No. 89. AHRQ Publication No. 11-05159-EF-1. Rockville, MD: Agency for Healthcare Research and Quality, 2011.

MARCUS, D. L.; STRAFACI, J. A.; FREEDMAN, M. L. Differential neuronal expression of manganese superoxide dismutase in Alzheimer's disease. Med. Sci. Monit. 12 BR8-14, 2006.

NICOLAU, A. Trends in the use of protozoa in the assessment of wastewater treatment. Res. Microbiol., v. 152, n. 7, p. 621-30, 2001.

OLINSKI, R.; JARUGA, P.; ZASTAWNY, T. H. Oxidative DNA base modifications as factors in carcinogenesis. **Acta. Biochim. Pol.,** v. 45, p. 561-572, 1998.

OZBEN, T. Oxidative stress and apoptosis: impact on cancer therapy. **J. Pharm. Sci.,** v. 96, n. 9, p. 2181-96, 2007.

PEREIRA, A. N. Effect of low-power laser irradiation on cell growth and procollagen synthesis of cultured fibroblasts. **Lasers Surg. Med.,** v. 31, p. 263-267.

REDDY, G. K. Comparison of the photostimulatory effects of visible He-Ne and infrared Ga-As lasers on healing impaired diabetic rat wounds. **Lasers Surg. Med.,** New York, v. 33, n. 5, p. 344-351, 2003.

SOUZA, L. L. Endonuclease IV and Exonuclease III are involved in the repair and mutagenesis of DNA lesions induced by UVB in Escherichia coli. Mutagenesis, v. 21, p. 125–130, 2006.

VASHEGHANI-FARAHANI, A. et al. The association between coronary arterial dominancy and extent of coronary artery disease in angiography and paraclinical studies. **Clin. Anat.,** v. 21, p. 519–523, 2008.

VINCK, E. et al. Evidence of changes in sural nerve conduction mediated by light emitting diode irradiation. **Lasers Med. Sci.** v. 20, n. 1, p. 35-40, 2005.

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## FIGURES

**Figure 1:** Suveillance fraction of E. coli AB1157 submited to red LED (RLED).

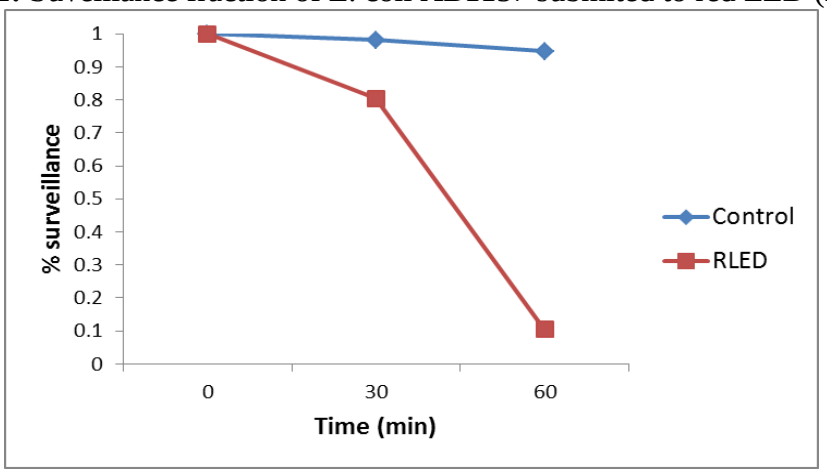


**Figure 2:** Suveillance fraction of E. coli AB1157 submited to green LED (GLED).

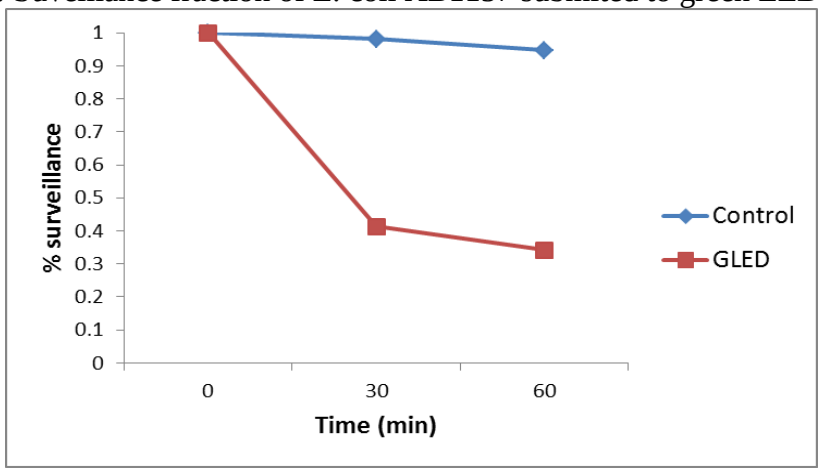


**Figure 3:** Suveillance fraction of E. coli AB1157 submited to blue LED (BLED).

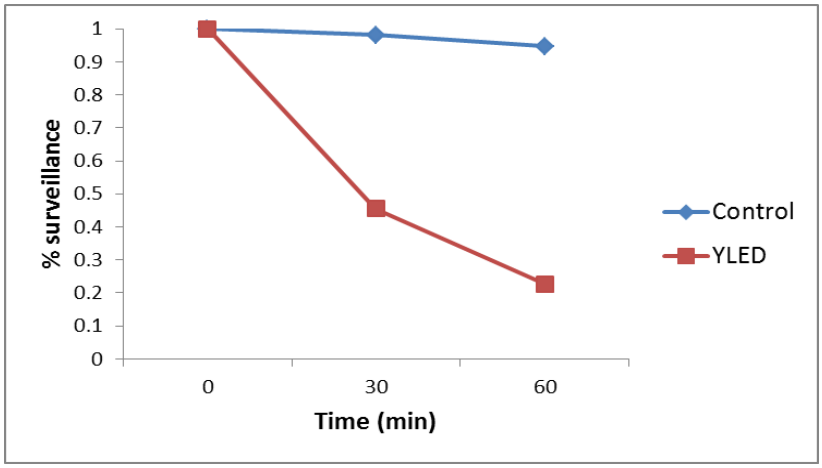

**Figure 4:** Suveillance fraction of E. coli AB1157 submited to yellow LED (YLED).

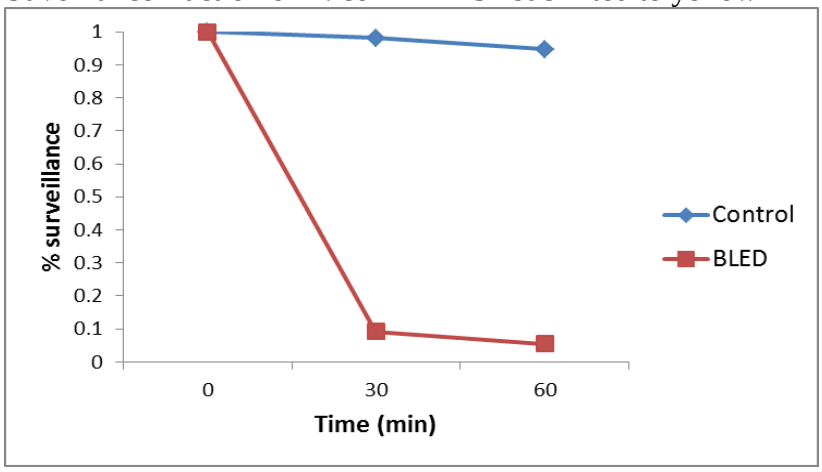


**Figure 5:** Surveillance fraction of E. coli BW9091 submitted to green LED (GLED).

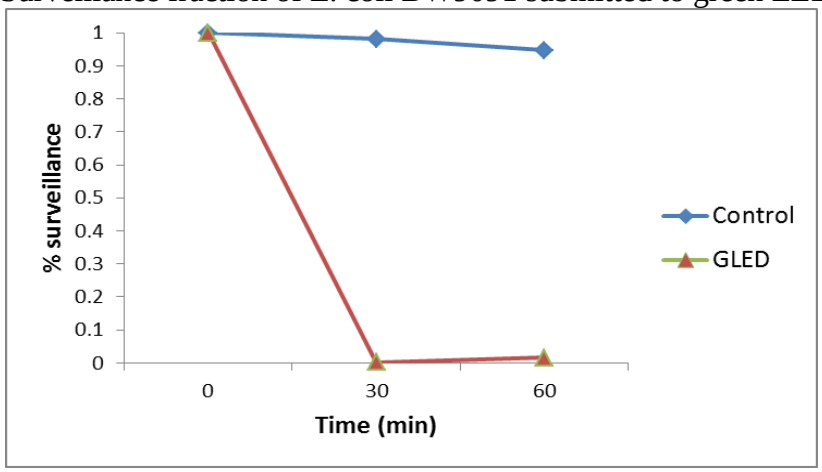


**Figure 6:** Surveillance fraction of E. coli BW9091 submitted to red LED (RLED).

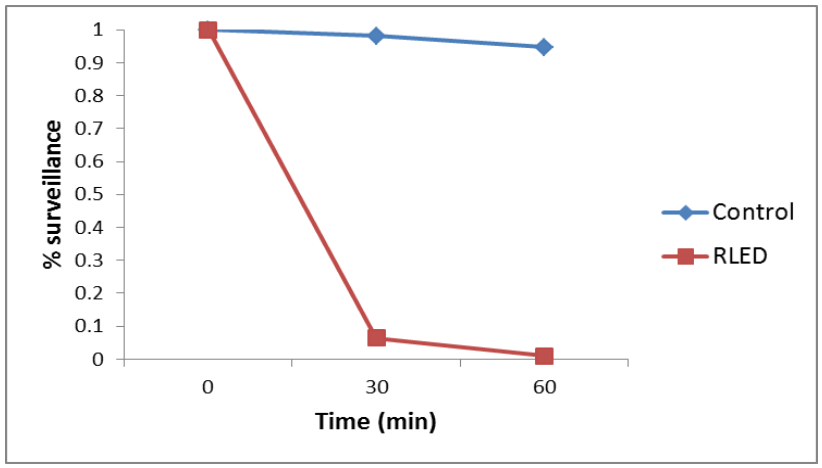


**Figure 7:** Surveillance fraction of E. coli BW9091 submitted to Yellow LED (YLED).

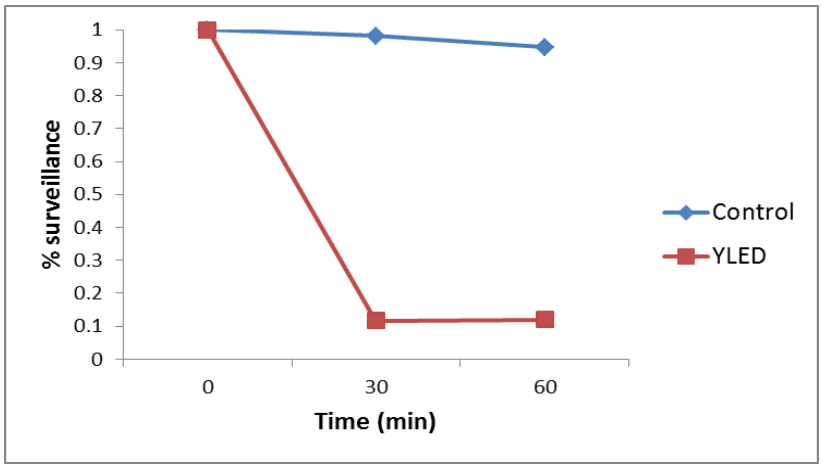


**Figure 8:** Surveillance fraction of E. coli BW9091 submitted to blue LED (BLED).