Websites:
http://www.nbmedicine.org
http://www.sciencepub.net/nurse

Emails:
editor@sciencepub.net
nbmeditor@gmail.com

Biomedicine and Nursing

MARSLAND PRESS
Multidisciplinary Academic Journal Publisher

APPLICATION OF BIOSYNTHESISED RIBOFLAVIN FROM *Bacillus subtilis* AG06 IN ENHANCED SOLAR DISINFECTION OF WATER SAMPLES

Ajunwa, O.M.[1] and Audu, J.O.[2*]

1. Department of Microbiology, Modibbo Adama University of Technology, Yola, Adamawa state, Nigeria
2. Department of Laboratory Technology, Modibbo Adama University of Technology, Yola, Adamawa state, Nigeria

*Corresponding Author: j3suwa@yahoo.com +2347037727211

ABSTRACT: The effects of solar radiation in disinfection of water have been activated as a growing technology in less developed areas of the world. One mechanism of solar disinfection has been attributed to cellular damage as a result of the alteration and intoxication induced by ultra violet (UV) rays on the respiratory systems inherent within the microbial cell envelopes. Riboflavin as a major factor in microbial cellular respiratory activities within the cell membrane has potentials of UV-coupled catalysis of cellular damage against water borne microorganisms in solar disinfection systems. *Bacillus subtilis* AG06 was used in biosynthesizing riboflavin using glucose minimal salt (GMS) medium. Highest riboflavin production of 90mg/L was obtained at culture conditions of 36h incubation, pH 7.2 and temperature 40°C. Different concentrations (30, 60, and 90mg/L) of biosynthesized riboflavin (at constant volume of 100ml) was applied in local solar disinfection set-ups using 1.5L polyethelene teraphtalate bottles containing clear water samples at varying times (2, 4 and 6 hours) starting from 10am. Microbial load of treated and untreated water samples was observed spectrophotometrically (at 600nm) and culturally (plating on Nutrient agar, MacConkey agar and Chloramphenicol-Yeast extract agar), showing a reduction in microbial life in treated samples and continuous growth in untreated samples. The highest values of reduction in total microbial life (up to 90%) were observed in set-ups supplemented with 100ml of 90mg/L riboflavin at 6h solar exposure. Riboflavin augmented solar disinfection systems have proven to be a simple means of enhanced domestic water disinfection for resource-poor settings.

[Ajunwa, O.M. and Audu, J.O. **APPLICATION OF BIOSYNTHESISED RIBOFLAVIN FROM *Bacillus subtilis* AG06 IN ENHANCED SOLAR DISINFECTION OF WATER SAMPLES.** *Biomedicine and Nursing* 2023;9 (2):53-60]. ISSN 2379-8211(print); ISSN 2379-8203(online). http://www.nbmedicine.org. 07. doi:10.7537/marsbnj090223.07.

Keywords: *Bacillus subtilis*, riboflavin, ultra violet rays, water disinfection

Introduction

Water is a key agent that supports humanity and is an everyday demand for the sustenance of life. According to the United Nations (UN, 2012), projections for 2015 show that 605 million people will still remain without access to quality source of drinking water. Approximately, this is a staggering 10% of the world population, thus showing the extent to which poor water quality burdens the entire planet especially manifest in the form of diarrhoeal infections and general poor health. Water disinfection mechanisms have been shown to aid in domestic reductions in water-borne infection rates (WHO/UNICEF, 2005, Esrey *et al*., 1991). A number of water disinfection methods have been practiced over the many years of water quality control and management. The household methods include filtration, boiling, chlorination and solar disinfection.

Solar disinfection is an age long practice with proven results. According to Baker (1981), the practice of solar disinfection dates back to over 2000 years ago; attributed to practices by local Indian communities who regarded it as a means for obtaining blessing of the sun on their water before consumption. In this regard, solar disinfection as a viable means of obtaining improved water quality has been the focus of some vigorous researches over the years (Acra *et al*., 1980; Acra *et al.,* 1989; Sommer *et al*., 1997; Reed, 1997; Mc Guigan *et al*., 1998; Joyce *et al*., 1992, Ubomba-Jaswa *et al*., 2010; Dejung *et al*., 2007). For an improvement of the efficacy of solar disinfection and designing of new methods of application, it is important to assess the mechanism of action against microorganisms, the major cause of water-borne diarrhoeal infections. Ultraviolet (UV) rays usually span a wavelength of 100-400nm and have direct effects on microbial DNA when irradiated causing formation of unnatural bonds

between pyrimidine bases on adjacent formations and subsequently leading to pyrimidine dimmers (Godsell, 2001) causing dysfunctional base pairing with purines of complementary DNA strands and thus changing the whole conformity of the DNA molecule at such points, leading to improper copying by DNA polymerases. In the case of solar disinfection which comes in the form of visible light, at a wavelength range of 315-400nm, the effect on microbial DNA bases is not directly damaging, but can indirectly lead to the formation of Reactive Oxygen Species (ROS) like hydrogen peroxide, hydroxyl radical and singlet oxygen in water. Upon formation of ROS, DNA damage is imminent as well as amino acid and fatty acid oxidation within the cells, which can be quite deleterious to cellular life (Reed *et al*., 2000). Previous researchers (Eisenstark, 1987; Tuveson and Sammartano, 1986; Sammartano and Tuveson, 1987), have determined the effect of microbial cellular respiratory cofactors as endogenous photosensitizing agents, quinone, porphyrin and flavin containing compounds have been identified as photosensitizing agents that can react with oxygen to produce ROS which can have a disinfecting action against water borne microbes (Jagger, 1981; Reed *et al*., 2000; Bosshard *et al*., 2010; Lonnen *et al*., 2005).

Riboflavin as a flavin containing agent of microbial respiration is an ideal photosensitizing candidate that can be applied in the improvement of solar disinfection of water samples. Lloyd *et al*. (1990) earlier reported the efficacy of riboflavin against *Escherichia coli* at UV wavelength of 320-400nm. It was also reported that introduction of riboflavin as an exogenous addition into a solar disinfection setup for water led to a major enhancement of the disinfection process against organisms like *Candida albicans*, *Escherichia coli* and *Fusarium solani* cells (Heaselgrave and Kilvington, 2010). Riboflavin is the precursor of coenzymes flavin mononucleotide (FMN) and flavin adenine dinucleotide (FAD), and can be biosynthesized by many plants and microorganisms, however, one of the most effective microbial source of riboflavin is the bacteria *Bacillus subtilis* (Lim *et al*., 2001) and its inherent riboflavin biosynthetic pathways have been very well elucidated (Vitreschak *et al*., 2002). This makes the bacteria an ideal candidate for easy industrial application in riboflavin synthesis.

The use of commercially synthesized riboflavin additives for water treatment can have its toll on the sustainability of the treatment process due to the process cost of chemical synthesis. It is then imperative that a more grass-root means of synthesizing riboflavin be applied in water disinfection. This work focuses on biosynthesizing riboflavin from *Bacillus subtilis* because of its efficiency and subsequently, its application in a simple water disinfection process.

Materials and Methods

Microorganism and Media

Bacillus subtilis AG05 obtained from earlier research work (Ajunwa *et al*., 2014) was used for this study. The isolate was grown and maintained in nutrient medium and subsequently propagated unto the production media - glucose minimal salt (GMS) medium with the following composition: Glucose - 10g/l, $(NH_4)_2SO_4$ – 2g, , KH_2PO_4 - 1g/l, K_2HPO_4 – 0.4g/l, $MgSO_4.7H_2O$ - 0.5g/l, $FeSO_4$ - 0.01g/l. Media was sterilized at 121ºC for 15 minutes

Riboflavin production and quantification

Riboflavin production was conducted following modifications to the procedure of Viashnavi and Daniel (2012) by inoculating two loopfuls of the isolate into the production media and incubating at 35ºC for 36h using pH 7.2. To quantify the amount of riboflavin produced, the procedure of Bartzatt and Wol (2014) was adopted. Standard solution of 0.5g/l of riboflavin (1.4 x 10^{-3} molar), mixed with sterile distilled water was prepared, and varying concentrations of this standard solution were constituted. The absorbance was determined using a UV-visible spectrophotometer (at 440nm), and standard plots of riboflavin concentration was used for estimating the riboflavin content of the test samples at different conditions. The percentage of riboflavin present in the sample was subsequently quantified.

Effects of physicochemical parameters on riboflavin yield

The effects of pH, temperature and incubation time on riboflavin yield were assessed. pH values of 4.2, 7.2 and 10.2 were tested. Test temperatures of 35ºC, 40ºC and 45ºC and incubation times of 24h, 36h and 48h were used for this study. For varied temperatures, pH value was maintained at 7.2 and incubation time at 36h. For pH variations, conditions were maintained at 40ºC temperature, and 36h incubation time. For incubation time variations, pH was maintained at 7.2 while temperature was 40ºC. Riboflavin yield was measured at the end of each test condition.

Application of biosynthesized riboflavin

The crude riboflavin product was obtained by centrifuging the production broths at 5000 rpm for 20 mins. The cell free liquid was applied in treatment of

clear domestic water samples in 1.5L PET bottles. Known volumes (100ml) of the biosynthesized riboflavin at varied concentrations (30, 60 and 90mg/L) were added in water samples and the samples were exposed to sunlight exposure on a clear, bright and warm day (average temperature of 39ºC) for varied times of 2, 4, and 6 hrs starting from 10am.. Samples not treated with riboflavin but exposed to solar radiation, and samples not treated with riboflavin and not exposed to solar radiation served as both positive and negative controls.

Measurement of antimicrobial effect in treated water samples

Antibacterial effects were determined by correlative quantification of microbial growth culturally and spectrophotometrically. The water samples at different exposure times were tested for their absorbance at 600nm. For cultural assessment, the samples were serially diluted, plated on Nutrient agar, MacConkey agar and Chloramphenicol-yeast extract agar and incubated at 35ºC for 18-24 h. The total viable bacterial count was determined by counting surviving colonies and subsequently correlated with absorbance values.

Results and Discussion

Quantification of riboflavin produced under different pH values showed that pH 7.2 favoured best riboflavin production with *B. subtilis* AG06. The best temperature was 40ºC, while an incubation time of 48h best supported riboflavin yield. Figures 1, 2 and 3 show the values of riboflavin concentration under different conditions. Riboflavin is a primary metabolite of *Bacillus subtilis*, thus its synthesis was correlated with optimum growth conditions of the organism. Temperature, pH, and incubation time optima exhibited by the isolate was in direct relationship with the range of values for functional growth of *Bacillus* species. Younis *et al*. (2010) reported a pH optimum range of 6.5-7, temperature optimum range of 40-45ºC, and best incubation periods of between 24-28 hours for *B. subtilis* strains tested. Korsten and Cook (1996) also corroborated that fact and reported an optimum incubation time for 24 hours onwards and temperature of 30-37ºC with maximum at 43ºC

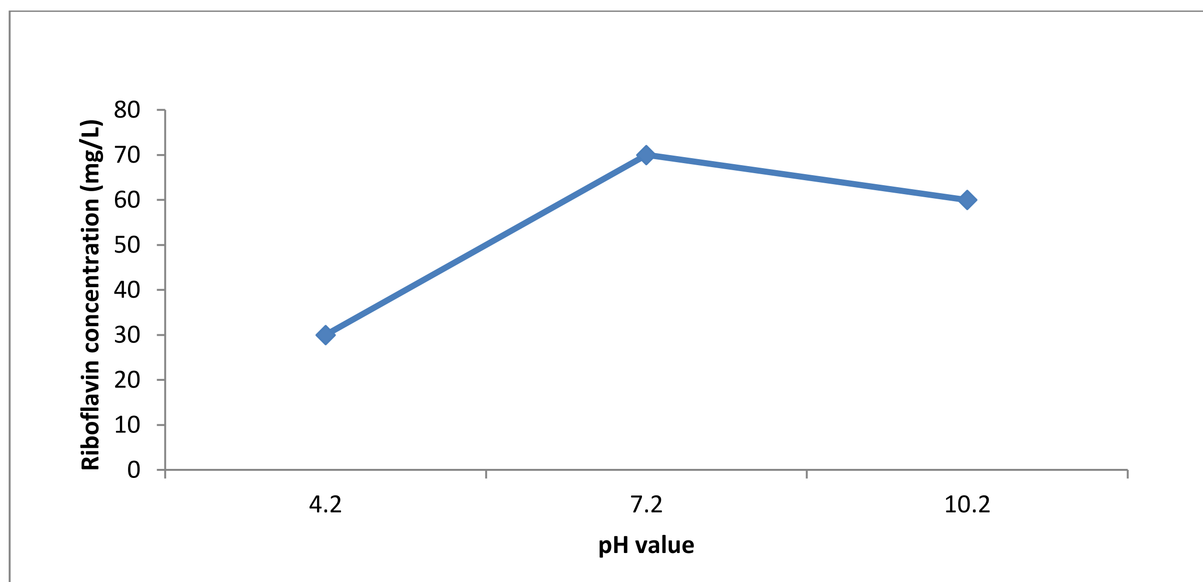

Figure 1: Riboflavin yield under different pH values

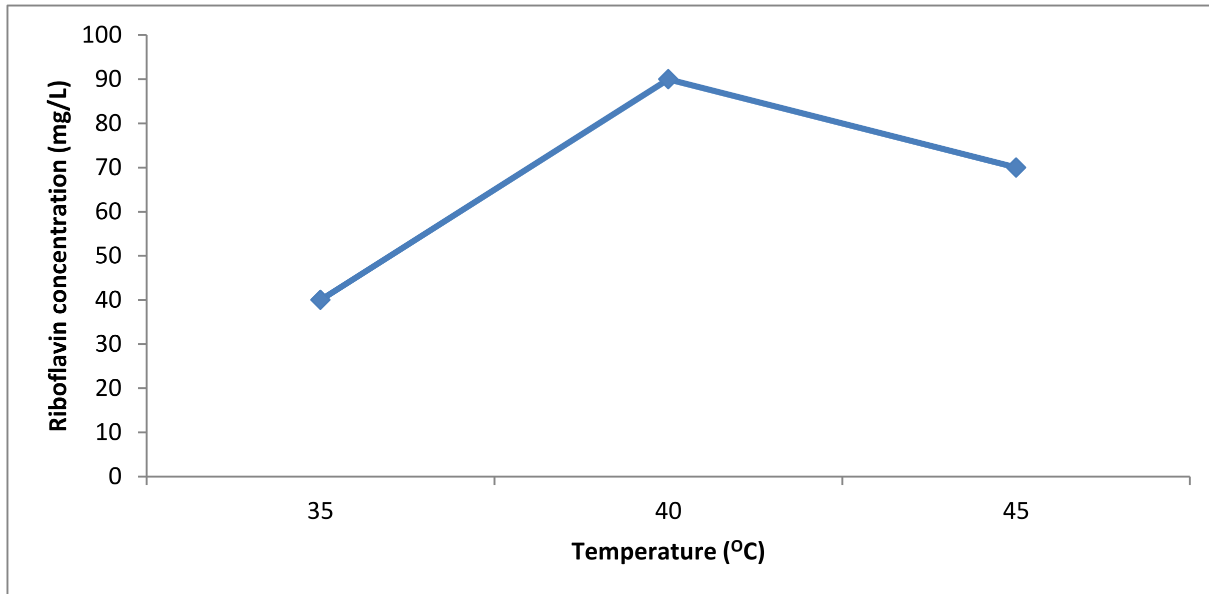

Figure 2: Riboflavin yield under different temperatures

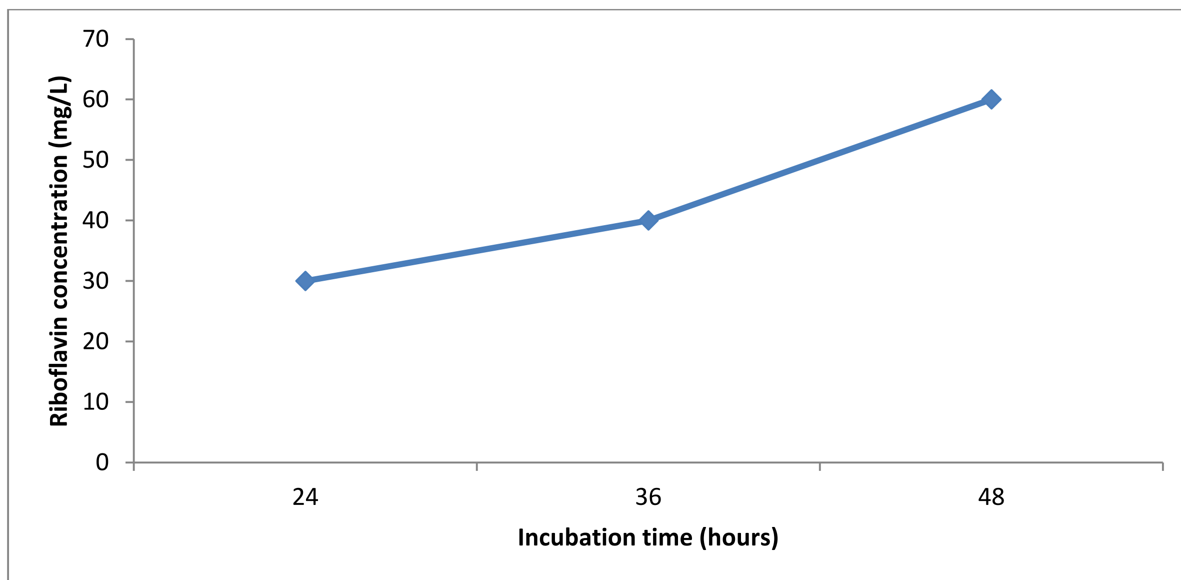

Figure 3: Riboflavin yield under different incubation times

At the end of the cultural and spectrophotometric assessments of bacterial growth, it was observed that there was a steady decline in bacterial growths as the exposure time increased. There was however an obviously evident difference in the bacterial cell number/load decline between the riboflavin treated samples and the untreated samples. The effect of riboflavin on solar water treatment was positive as it led to higher decline in microbial load within the water samples as showed by both the cultural and the spectrophotometric readings. Tables 1,2 and 3 showed the decline in microbial load based on the different media used in assessing microbial growth. The functionality of the media was to aid in general microbial growth of all bacterial pathogens (using nutrient media), selective growth of coliforms (using mac conkey media) and selective growth of fungi (using chloramphenicol-yeast extract media). Inactivation and elimination of microbial life was observed in all water samples tested, including the control (without addition of ribloflavin) however, there was marked reduction in the riboflavin augmented test systems.

Table 1: Microbial load (on nutrient agar) of concentration based riboflavin-augmented water samples with varied solar exposure times

Riboflavin concentration	**CFU/ml of microbial growth on nutrient agar at different exposure times**			
	0hour	2hours	4hours	6hours
30mg/L	$5.0x10^{8}$	$3.0x10^{6}$	$2.7x10^{4}$	2.0×10^{2}
60mg/L	$4.7x10^{8}$	$3.7x10^{4}$	$6.1x10^{3}$	1.3×10^{2}
90mg/L	$6.2x10^{8}$	$3.3x10^{3}$	$1.1x10^{2}$	62
Control	$5.2x10^{8}$	$3.2x10^{7}$	$6.0x10^{5}$	$3.1x10^{3}$

Table 2: Microbial load (on mac conkey agar) of concentration based riboflavin-augmented water samples with varied solar exposure times

Riboflavin concentration	**CFU/ml of microbial growth on mac conkey agar at different exposure times**			
	0hour	2hours	4hours	6hours
0.3g/L	$4.0x10^{6}$	$2.5x10^{4}$	$1.1x10^{2}$	60
0.6g/L	$4.7x10^{6}$	$3.7x10^{3}$	710	88
0.9g/L	$5.6x10^{6}$	$6.3x10^{2}$	553	39
Control	$5.4x10^{8}$	$4.1x10^{7}$	$6.8x10^{5}$	$7.3x10^{3}$

Table 3: Microbial load (on chloramphenicol-yeast extract agar) of concentration based riboflavin-augmented water samples with varied solar exposure times

Riboflavin concentration	**CFU/ml of microbial growth on chloramphenicol-yeast extract agar at different exposure times**			
	0hour	2hours	4hours	6hours
30mg/L	4.4×10^{4}	5.0×10^{2}	3.1×10^{1}	30
60mg/L	4.7×10^{4}	90	41	-
90mg/L	2.8×10^{4}	42	33	-
Control	8.4×10^{8}	2.9×10^{7}	8.4×10^{5}	3.3×10^{3}

With respect to bacterial inactivation within solar disinfection setups, *Escherichia coli* a highly genomically characterized bacterium and also a major indicator of fecal contamination of water, formed the crux of some earlier studies on bacterial inactivation by solar disinfection. Webb and Brown (1979) reported a ultraviolet wavelength and oxygen dependent inactivation of *E. coli* cells, while Reed (1997) reported an inactivation in solar treated *E. coli* cells based on the effects of dissolved oxygen. Berney *et al.* (2006) adopted flow cytometric studies to determine the inactivation of *E. coli* by solar disinfection. It was shown that solar disinfection caused a disruption in sequence of normal cellular functions including ATP synthesis and efflux pump activity, as well as a progressive loss of membrane potential and reduced glucose uptake culminating in permeability of the cytoplasmic membrane, loss of culturability and subsequent death of cells. The solar exposure time also influenced gradual cellular decline, as more effective reduction in microbial loads was observed at higher exposure times with highest reduction at 6 hours exposure. Basically, most water borne bacteria have been proven to be negatively affected by solar disinfection mechanisms after exposure to about 6 hours of exposure under suitable conditions (Dejung *et al*., 2007, Waeglin *et al*., 1994)

Inactivation of fungi by the solar experimental set-up was also observed in this study, with maximum reduction of up to 100% observed at 6h exposure times with 60mg/l and 90mg/l riboflavin augmented setups. Cellular activation of *Candida albicans* in water samples using solar disinfection was reported by Lonnen *et al.* (2005) and Sichel *et al*. (2007) at 6 hours of exposure. Other works on the effects of solar

disinfection on *Fusarium* species showed a specie-variable decline of *Fusarium* under natural solar light as a function of ultraviolet energy per unit volume in kilo Joules per liter (KJ/L) (Fernandez-Ibanez *et al.*, 2009).

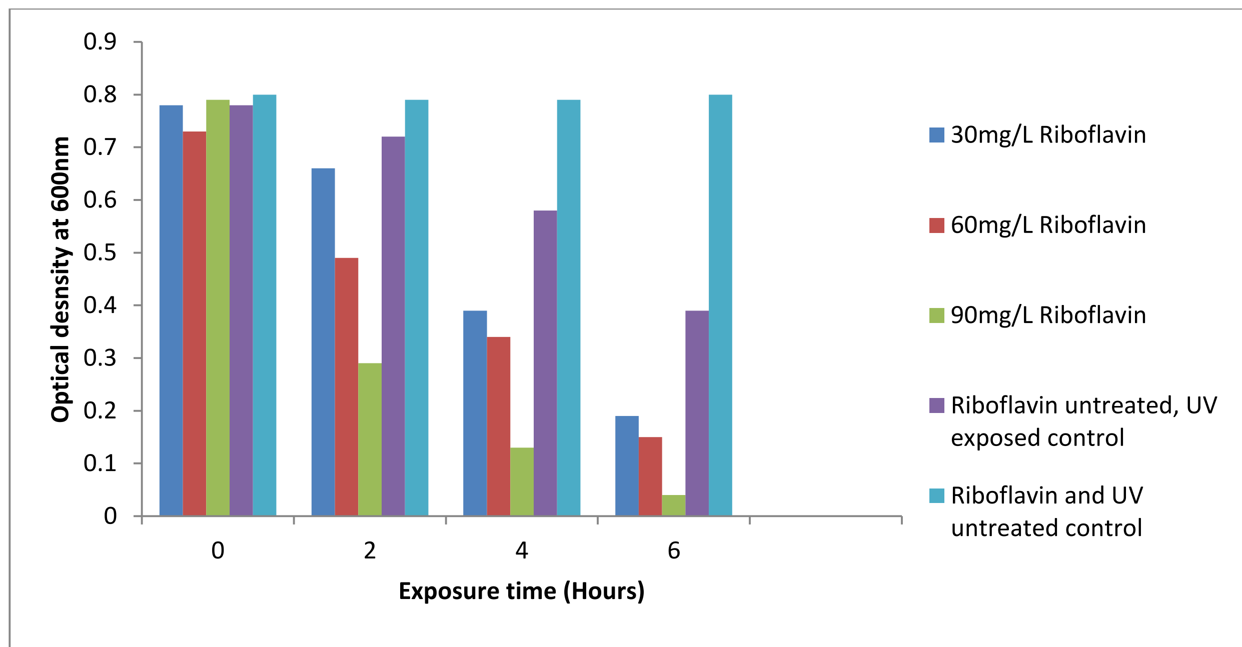

Figure 4: Optical density of water samples treated with different concentrations of riboflavin and exposed to solar radiation at different exposure times.

From figure 4, the riboflavin concentrations when increased made more impact in disinfection of the samples, and reduction in microbial life. The least decline was observed with the untreated samples, while the highest growth decline of up to 90% was observed for the setup augmented with 90mg/L riboflavin and exposed to solar radiation for up to 6hours, showing the effect of riboflavin in inactivation of microbial contaminants of water. The effect of riboflavin was also similar in a test carried out by Heaselgrave and Kilvington (2010) as it enhanced solar disinfection against bacterial, fungal and protozoan pathogens. The effects of riboflavin are determined as photocatalytic and can lead to the formation of reactive oxygen species (ROS) within the water which can be highly deleterious to microbial life (Blake *et al.*, 1999). From the riboflavin untreated samples which was exposed to solar radiation, there was visible time dependent reduction in growth, owing to the effects of ultraviolet rays alone, however, it was less effective than the riboflavin aided samples. Thus a riboflavin augmented solar disinfection system for water samples was far more efficient than a system without riboflavin.

Conclusion

In conclusion, solar disinfection methods for water treatment are a very cheap alternative to the several other methods proposed. It is very energy efficient and user friendly with little manipulative skills required. With the proven abilities of this form of water disinfection to reduce drastically all forms of microbial life, it stands as a viable tool for the reduction of rates of infantile dysentery and diarrhea, as well as other water related microbial infections, that ave plagued less developed areas of the world which our country falls under. Research on simple methods of upgrading and enhancing this disinfection process are very welcome, and further sensitization programmes and enlightenment campaigns to promote the concepts of this promising technology are advocated.

REFERENCES

[1]. Bartzatt, R. and Wol, T. (2014) Detection and Assay of Vitamin B-2 (Riboflavin) in Alkaline Borate Buffer with UV/Visible Spectrophotometry. *International Scholarly Research Notices,* Volume 2014, Article ID 453085, 7 pages. http://dx.doi.org/10.1155/2014/453085

[2]. Younis, M.A.M., Hezayen, F.F., Nour-Eldein, M.A. and Shabeb, M.S.A. (2010) Optimisation of Cultivation Medium and Growth Conditions for *Bacillus subtilis* KO Strain Isolated from Sugar

Cane Molasses. *American-Eurasian J. Agric. Envir. Sci.* 7(1): 31-37

[3]. Korsten, L. and Cook, N. (1996). Optimising Culturing Conditions for *Bacillus subtilis. South African Avocado Grower's Association Yearbook* 19:54-58

[4]. Ajunwa, O.M., Odeniyi, O.A., Adeleke, A.J., Orji, F.A., Ewansiha, J.U. and Obidah, J.S. (2014). Applicaation of Polyurethane foam matrix as immobilizer in the production of Thermostable Alkaline Protease by *Bacillus subtilis* isolated from barbeque spots. *J. of Biol. Sci.* 14 (7): 472-479.

[5]. Lim, S.H., Choi, J.S. and Park, E.Y (2001). Microbial Production of Riboflavin Using Riboflavin Overproducers, *Ashbya gossypii*, *Bacillus subtilis*, and *Candida famate:* An Overview . *Biotechnol. Bioprocess Eng.* 6: 75-88

[6]. UN, The Millennium Development Goals Report (2012) ISBN 978-92-1- 101258-3.

[7]. W.H.O./U.N.I.C.E.F., WATER FOR LIFE – Making it happen, Joint Monitoring Programme for Water Supply and Sanitation, ISBN 92-4-1562-935 (2005).

[8]. Esrey, S.A., Potash, J.B., Roberts, L. and Shiff, C. (1991). Effects of improved water supply and sanitation on ascariasis, diarrhoea, dracunculiasis, hookworm infection, schistosomiasis, and trachoma. *Bull. World Health Organisation* 69: 609–621

[9]. Sommer, B., Marino, A., Solarte, Y., Salas, M.L., Dierolf, C., Valiente, C., Mora, D., Rechsteiner, R., Setter, P., Wirojanagud, W., Ajarmeh, H., AlHassan, A. and Wegelin, M. (1997). SODIS – an emerging water treatment process, *J. Water SRT – Aqua* 46: 127–137.

[10]. Reed, R.H. (1997). Solar inactivation of faecal bacteria in water: the critical role of oxygen. *Lett. Appl. Microbiol*. 24: 276–280.

[11]. Baker, M.N.T.M. (1981). The Quest for Pure Water: The History of the Twentieth Century, *AWWA*.

[12]. Acra, A., Jurdi, M., Mu'Allem, H., Karahagopian, Y. and Raffoul, Z. (1989). Sunlight as disin-fectant. *Lancet* 1: 280.

[13]. Acra, A., Karahagopian, Y., Raffoul, Z. and Dajani, R. (1980). Disinfection of oral rehydration solutions by sunlight. *Lancet* 2: 1257–1258

[14]. Joyce, T., Kenny, V., McGuigan, K. and Barnes, J. (1992). Disinfection of water by sunlight. *Lancet* 340: 921 (Letter).

[15]. McGuigan, K.G., Joyce, T.M., Conroy, R.M., Gillespie, J.B. and Elmore-Meegan, M. (1998). Solar disinfection of drinking water contained in transparent plastic bottles: characterizing the bacterial inactivation process. *J. Appl. Microbiol.* 84: 1138–1148

[16]. Vaishnavi, M. and Daniel, D.K. (2012). The effect of environmental parameters on Vitamin B2 Production from Hydrocarbons by *Aspergillus awamori* NRRL 3112. *International Journal of Pharma Sciences and Research* 3: 279-286

[17]. Wegelin, M. Canonica, S. Mechsner, K. Fleischmann, T. Pesaro, F. and Metzler, A. (1994). Solar water disinfection: scope of the process and analysis of radiation experiments. *J. Water SRT – Aqua* 43:154–169.

[18]. Goodsell, D.S. (2001). The molecular perspective: ultraviolet light and pyrimidine dimmers, *Oncologist* 6: 298–299.

[19]. Eisenstark, A. (1987). Mutagenic and lethal effects of near-ultraviolet radiation (290- 400 nm) on bacteria and phage. *Environ. Mol. Mutagen*. 10: 317– 337.

[20]. Jagger, J. (1981). Near-UV radiation effects on micro-organisms. *Photochem. Photobiol.* 34: 761–768.

[21]. Lloyd, R.E. Rinkenberger, J.L. Hug, B.A. Tuveson, R.W. (1990). Growing *Escherichia coli* mutants deficient in riboflavin biosynthesis with nonlimiting riboflavin results in sensitization to inactivation by broad-spectrum near-ultraviolet light (320-400 nm), *Photochem. Photobiol*. 52: 897–901.

[22]. Sammartano, L.J. and Tuveson, R.W. (1987). *Escherichia coli* strains carrying the cloned cytochrome d terminal oxidase complex are sensitive to near-UV inactivation. *J. Bacteriol*. 169: 5304–5307.

[23]. Tuveson, R.W. and Sammartano, L.J. (1986). Sensitivity of hema mutant Escherichia coli-cells to inactivation by near-UV light depends on the level of supplementation with delta-aminolevulinic-acid. *Photochem. Photobiol*. 43:621– 626.

[24]. Webb, R.B. and Brown, M.S. (1979). Action spectra for oxygen-dependent and inde-pendent inactivation of Escherichia coli-Wp2s from 254-nm to 460-nm. *Photochem. Photobiol*. 29: 407–409.

[25]. Reed, R.H. Mani, S.K. and Meyer, V. (2000). Solar photo-oxidative disinfection of drinking water: preliminary field observations. *Lett. Appl. Microbiol*. 30: 432–436

[26]. Blake, D.M., Maness, P.C., Huang, Z., Wolfrum, E.J. and Huang, J. (1999). Application of the photocatalytic chemistry of titanium dioxide to disinfection and the killing of cancer cells. *Sep. Purif. Method* 28: 1-50

[27]. Whitelam, G.C. and Codd, G.A. (1982). A rapid whole-cell assay for superoxide dismutase, *Anal. Biochem.* 121: 207–212.
[28]. Bosshard, F. Bucheli, M. Meur, Y. and Egli, T. (2010). The respiratory chain is the cell's Achilles' heel during UVA inactivation in *Escherichia coli*. *Microbiology* 156: 2006–2015.
[29]. Berney, M. Weilenmann, H.U. Egli, T. (2006). Flow-cytometric study of vital cellular functions in *Escherichia coli* during solar disinfection (SODIS), *Microbiol. Mol. Biol. Rev*. 152: 1719–1729.
[30]. Ubomba-Jaswa, E. Fernández-Ibá˜nez, P. Navntoft, C. Polo-López, M.I. and McGuigan, K.G. (2010). Investigating the microbial inactivation efficiency of a 25 litre batch solar disinfection (SODIS) reactor enhanced with a compound parabolic collector (CPC) for household use, *J. Chem. Technol. Biotechnol.* 85: 1028–1037.
[31]. Dejung, S. Fuentes, I. Almanza, G. Jarro, R. Navarro, L. Arias, G. Urquieta, E. Torrico, A. Fenandez, W. Iriarte, M. Birrer, C. Stahel, W.A. and Wegelin, M. (2007). Effect of solar water disinfection (SODIS) on model microorganisms under improved and field SODIS conditions, *J. Water SRT – Aqua* 56: 245–256.
[32]. Lonnen, J. Kilvington, S. Kehoe, S.C. Al-Touati, F. and McGuigan, K.G. (2005). Solar and photocatalytic disinfection of protozoan, fungal and bacterial microbes in drinking water. *Water Res*. 39: 877–883.
[33]. Sichel, C. de Cara, M. Tello, J. Blanco, J. and Fernández-Ibá˜nez, P. (2007). Solar photocatalytic disinfection of agricultural pathogenic fungi: *Fusarium* species, *Appl. Catal. B.* 74: 152–160
[34]. Sichel, C. Tello, J. de Cara, M. and Fernandez-Ibanez, P. (2007). Effect of UV solar intensity and dose on the photocatalytic disinfection of bacteria and fungi, *Catal. Today* 129: 152–160.
[35]. Heaselgrave, W. and Kilvington, S. (2010). Antimicrobial activity of simulated solar disinfection against bacterial, fungal, and protozoan pathogens and its enhancement by riboflavin. *Appl. Environ. Microbiol.* 76: 6010–6012
[36]. Fernández-Ibá˜nez, P., Sichel, C., Polo-López, I., de Cara-García, M. and Tello, J.C (2009) Photocatalytic disinfection of natural well water contaminated with *Fusarium solani* using TiO2 slurry in solar CPC photo-reactors. *Catal. Today* 144: 62–68.
[37]. Lonnen, J., Kilvington, S., Kehoe, S.C., Al-Touati, F. and McGuigan, K.G. (2005). Solar and photocatalytic disinfection of protozoan, fungal and bacterial microbes in drinking water. *Water Res*. 39: 877–883.
[38]. Vitreschak, A.G., Rodionov, D.A., Mironov, A.A. and Gelfand, M.S. (2002). Regulation of riboflavin synthesis and transport genes in bacteria by transcriptional and translational attenuation. *Nucleic Acids Res.* 30: 3141-3151

6/15/2023