

Enhancing the Performance of Solar Water Disinfection with Potassium Persulfate: Laboratory Study with *Enterococcus faecalis*

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Background & Aims of the Study: The safe drinking water providing is one of the most crucial objections in these centuries. Bacterial water contamination and high rate of morbidity and mortality is crucial health threat. Efficiency of potassium persulfate (KPS) associated solar disinfection as a novel water disinfection technology was evaluated in batch scale experiments, using *Ent. faecalis* (ATCC 29212).

Material and Methods: This research is a descriptive and experimental study which done on Tehran city, Iran. *Ent. faecalis* (ATCC 29212) was provided in standard form from reference laboratory. Desired bacterial density in water was prepared by Mc Farland method. Water specimens were exhibited with solar radiations from 10 a.m to 16 p.m of Tehran local time. All experiments were conducted into 1.5 L volume of Damavand bottled water. Non-injured bacteria cells were detected by plating onto Bile Esculin azide agar media. Turbid water samples were provided by spiking of sterile slurry. Contact time (1-6 h), turbidity (30-200 NTU), KPS concentration (0.1, 0.7, 1.5 and 2 mMol/l), *Ent. faecalis* density (1000 and 1500 cell/ml) and UV intensity were independent and disinfection efficiency was a dependent variable, respectively.

Results: Intensity of UVA solar irradiation varied from 3770 to 6263.3 $\mu\text{W}/\text{Cm}^2$, with the highest value was measured on 13.30 p.m. In single SODIS and 1 hour contact time, increasing of bacterial closeness from 1000 to 1500 cell/ml implied disinfection performance decreasing in which, the vital bacteria was 10 and 20 cell/ml, respectively; but beyond of this contact time, a complete disinfection was occurred. Disinfection of *Ent. faecalis* was achieved within 2 h with single solar irradiation but KPS associated solar disinfection with applied dosage of KPS provide completely disinfection in 1 h in which the process efficiency was not influenced by increasing of bacterial density and turbidity up to 200 NTU.

Conclusion: Association of KPS with SODIS enhancing water disinfection which can be used in remote area and emergency status.

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Background

Burden of water borne diseases due to their mortality and morbidity is a crucial health problem in this century (1,2). Waterborne dysentery is the second origin of fatality in population under five years old; in addition to the high fatality rate which estimated over than

two million, annually. It leads to malnutrition, with the relevant influences on physical maturation and susceptibility to other septicities (1). Also, diarrheal illness is the third cause of fatality in low-income communities, leads to evaluated 1.4 million mortality in recent years. Hence, water and food borne of pathogens including bacterial, viruses and protozoan via the faecal-oral rout are primarily concern.

WHO and UNICEF co reported estimation revealed over than one billion multinational people trust on water resources which have faecal contamination risks (3). Despite well progress of the Millennium Development Goal (MDG) target 2015 deadline, over than 783 million of people are still without the invulnerable potable water and billions without sanitation infrastructure (4). Stockholm International Water Institute reported that 50% of all hospital beds are tenanted by patients suffering from water-borne diseases, therefore, specially in remote and low-income area household water treatment via suitable techniques has been one of the most beneficial ways to eliminate the prevalence of water transmitted diseases (5).

Ent. faecalis are raised in high densities in human wastes. Due to their universality in human excreta and surveillance in the environment, enterococci have been confirmed as index of human feces contamination in water (6). Estimations implied that there are 800,000 cases of enterococcal disorders in the US, annually, adding \$500,000,000 to annual health services costs. So, the quality control of drinking and recreational waters for enterococci contamination is importantly considered. In the EU, enterococci are not licensed in a 100 mL sample of inspected potable water that flows from a valve and they are not accepted in a 250 mL specimen of bottled water (7). Several conventional techniques had been used for water disinfection but there have some drawbacks from point of applicability in remote area and formation of harmful disinfection byproducts which are suspected of being carcinogenic that 600 types of these compounds can be formed (8,9). In regions with high solar intensity and long sunny days solar disinfection (SODIS), may be a substitute, inexpensive and efficient method for drinking water expurgation at the domicile scale (10,11). In SODIS, the portion of UV-A, 315-400nm, discernible violet and blue light in the length of 400-490 nm of solar radiation generates antimicrobial activity.

Also, increasing of water temperature known have synergistically effects to future enhancing in SODIS studies demonstrated that the dysentery prevalence of children less than 5 years was eliminated by performance (12). More than 2 million inhabitants in 31 nations are using SODIS for their drinking water disinfection; so, 24% to 40% of diarrhea was eliminated through consumption of SODIS treated water (13). Despite its advantages, it suffers from relatively long disinfection time (from 2- 6 h) or for 2 consecutive days in the cloudy sky, and is affected by the solar radiations intensity and water unclarity (up to 30 NTU) (1,14). In this process, activating of dissolved organic carbon leads to the creation of reactive oxygen species. Since this process is over than 1,000-fold passive than immediate destruction of UV-C, several investigations have conducted to SODIS improvement, using riboflavin, TiO₂, H₂O₂ and copper plus ascorbic acid (5). Due to limitations of these compounds, the application of soluble compounds including potassium persulfate (KPS) was considered. Redox likely of the major composition of KPS (S₂O₈²⁻) is 2.05v which its activation by heat and UV range causes to the formation of the energetic sulfate cardinals (SO₄^{•-}) with redox power of 2.6 v that is conveyed via OH[•] cardinals, can lead to enhanced disinfection (2).

Aims of the study:

Accordingly, this study investigated KPS associated SODIS performance for *Ent. faecalis* disinfection for providing of the safe drinking water in remote, low-income and field applications.

Materials & Methods

Materials and equipment's

This experimental and descriptive research was performed in Tehran (Longitude 51° 2' to 51° 36' E and Latitude 35° 34' to 35° 50' N, Sea level 1110 m). The observations were carried out on the crown of a construction, adjusted to the UV analyzing equipment's and laboratories for

microbial testing, as well the interferences of reversing or obscurities protected by the neighboring structures was at the lowest status. Powerity of UV light was measured by UVA analyzer (UV LIGHT METER UVA-UVB 290 nm-390 nm- Lutron Taiwan). KPS was provided from Merck Company with presented identifications in Table 1 and Fig.1; spiked on water without absolution in 0.1, 0.7, 1.5 and 2 mMol/l. The water samples were exhibited with sun radiations from 10 a.m to 16 p.m (6 h) of local time. Fluctuation of total water components from points of liquefied solids and sulfate concentrations, which is relevant with KPS spiking was determined by recommended standard procedures (15).

Table 1) Physical and chemical properties of potassium persulfate (16)

Property	Value
Physical state	Solid
Melting point	Decomposes @ ca. 100 °C
Relative density	2.48 g/cm ³ @ 20 °C
Water solubility	60 g/l @ 25 °C
pH	5-8 for 1% solution
Oxidizing properties	Oxidizer

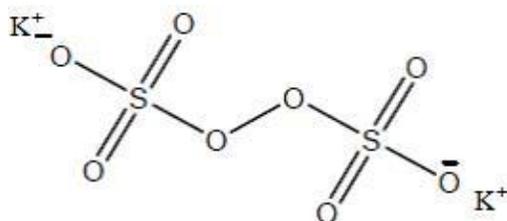


Figure 1) Structural formula of potassium persulfate (KPS)

All observations were performed in food grade low-density PET bottles of 1.5 L volume (Damavand IR.) The bottles compactness was 45µm in which the *Ent. faecalis* suspension injection and sampling for bacterial culture was conducted by screw top plastic with 40 mm diameter. The observations were performed on

summer season days, related to a calendar date from 15 of July to 15 of august, 2013. The experimental equipment's form is presented in Fig. 2.



Figure 2) SODIS and KPS associated SODIS reactors configuration

***Enterococcus faecalis* growth and working suspension**

Ent. faecalis (ATCC 29212) was chosen as a faecal pollution index which provided from reference laboratory. The standard and lyophilized form of *Ent. faecalis* was pended by injection of sterile nutrient broth medium and stockpiled at 4°C. A separated colony of *Ent. faecalis* was supplied by the vaccination in nutrient agar medium and incubation in 37°C for 24 h. Required bacterial concentration was provided by inoculation of colonies in BHI broth (Incubated-shaked in 37°C for 24±2 h). The suspension was separated in 3000 rpm for 10 min and rinsed with physiological serum (0.01%). The dense of bacterial suspension in serum was measured with photometer (Cecil-1011) in 625 nm wavelength. Based on light transmission the bacterial concentration of this suspension was 0.5 Mac Farland (1.5×10⁸ cell/ml) which is the required bacterial concentration in water (1000 and 1500 cell/ml) formulated by injection of 6.6 and 10 µl of *Ent. faecalis* suspension onto Damavand bottled water. The identification and growth of non-injured bacterial cells was conducted by samples culturing onto Bile Esculin agar media (Merck Co). The disinfected culture media were cascaded in plates and hoarded at 4°C (no more than 48 h). Survey of residual *Ent. faecalis* was performed by serial rarities of samples were injected on the BHI agar plate,

followed via reproduction for 24±4 h at 37°C and enumeration of visibly diagnosed *Ent. faecalis* colonies (17).

Preparation of Muddy Water Samples

Muddy water samples were formulated based on our previous work (2). Briefly, 5-10 g of decontaminated soil via heating (120 °C for 2 h) was added to 1 L of distilled water for providing of decontaminated suspension (15 min, 121°C and 1.5 bars). The suspension was disturbed for 15 min and then reserved for establishing for 30 min. The muddy water with fine composing were centrifuged and appropriate water turbidity (30, 50, 100, 150 and 200 NTU) determined with a standard turbidimeter (DRT-15CE) was regulated by infusion of desire volume of the muddy water (2).

Results

Chemical quality of Damavand bottled water which used in this research was determined by quality assurance ward of this company and analysis of some components in laboratory based on standard methods for water and waste water hand book guidance (Table. 2) Also, analysis of disinfected water quality from points of pH and temperature by SODIS associated with KPS revealed that these parameters of disinfected water are in accordance with Iranian drinking water guidelines (Tem. 39.85±2 and pH=7.4±0.3).

Table 3 shows the effects of bacterial density and contact time on single SODIS disinfection (without KPS) performance. Based on Tab. 3, although, increasing of *Ent. faecalis* led to decreasing of disinfection performance but based on WHO classification this technique can lead to enhancing of water quality from very hazard to low hazard status and decreasing of *Ent. faecalis* contamination risks. Tab.3 implied water quality assurance from point of *Ent. faecalis* disinfection; the minimum contact time of 2 h is necessary.

The results of Paveh schools environmental health and safety evaluation showed, under the terms of regulations and checklist, from the 28 visited schools 35.6% of schools building were old and 63.7% of schools building were new built (table 1). In terms of environmental health standards in 20 schools environmental health status were desirable and in 6 schools semi-desirable and in 2 schools were undesirable. And in term of safety status 21 schools were desirable and 6 schools were semi-desirable in safety condition (table 2). On this basis status of toilet and washbasins, sewage and garbage disposal in all schools were healthy and had good brightness and 93% of schools had healthy drinking fountains and 96.4% of schools had inadequate green spaces. Other results separately are described by charts (Figure 1, 2).

Table 2) Chemical quality of water used in experiments

Component	Unit	Concentration
Ca ⁺⁺	mg/l	78.5
Mg ⁺⁺	mg/l	18.7
Na ⁺	mg/l	6.2
K ⁺	mg/l	0.8
HCO ₃ ⁻	mg/l	280
SO ₄ ²⁻	mg/l	20
NO ₃ (N)	mg/l	1.8
F	mg/l	0.2
SiO ₂	mg/l	13.2
pH	-	7.2
Temperature	20	°C

The enhanced effects of KPS association in disinfection process with different *Ent. faecalis* density were shown on Tab 4 and 5. These results revealed that KPS dosage had not significant effects on disinfection performance. Also, the presence of KPS overcomes bacterial density effects. So, the application of KPS with SODIS led to enhanced disinfection efficiency in which the required contact time decreased up to 2 fold. Therefore, in 1 h contact time, 1000

and 1500 CFU/ml of *Ent. faecalis* and single SODIS process the non-injured residual bacteria are 10 and 20 CFU/ml, respectively (Tab. 3), but in KPS associated SODIS completely disinfection was occurred within 1 h (Tab. 4 and 5); so, based on WHO classification the safe drinking water was provided.

Tables 3 and 4 show that in 1000 CFU/ml of *Ent. faecalis*, same intensity of UV irradiation and different dosage of KPS, disinfection accelerated and KPS associated system implied high performance.

Based on Tab. 3 to 5, it can be concluded that KPS association can lead to enhanced, accelerated and completely disinfection which can be occurred within 1 h contact time and 0.1 mMol/l of KPS. Survey the effects of turbidity on water disinfection revealed that in single SODIS and KPS associated SODIS with different KPS dosages, elevation of water turbidity up to 200 NTU had not influenced the disinfection efficacy and in both processes completely disinfection was performed (data not shown).

According to KPS dissociation in aqueous solution to SO_4^{2-} and K^+ , determining of these ions and total dissolved solids (TDS) concentration as disinfection by-products of KPS spiking on water is necessary. Monitoring of disinfected water quality implied that spiking of KPS up to 2 mMol/l had not depletion effects on water quality from point of TDS and SO_4^{2-} as critical components. Since, in this study 0.1 mMol/l of KPS was determined as an optimum dosage of disinfection enhancer, so, it can be claimed that association of KPS in SODIS process has not drawbacks and leads to enhanced disinfection. Therefore, the disinfected water quality with this process is in accordance with the WHO and Iranian drinking water standards. Several previous studies reported that SODIS process suffers from bacterial regrowth. In this study, *Ent. faecalis* regrowth was evaluated in disinfected water after 24 h, 48 h and one week. The results show that all of water samples were negative from the point of *Ent. faecalis* presence.

Table 3) Effects of *Ent. faecalis* density and contact time on single SODIS performance

<i>Ent. faecalis</i> Density	Contact time(h)					
	1	2	3	4	5	6
1000 CFU/ml	10	0	0	0	0	0
1500 CFU/ml	20	0	0	0	0	0
Solar UV intensity($\mu\text{W}/\text{Cm}^2$)	3837.5	5157.5	6263.3	6000	4926.7	3770

Table 4) The effect of contact time on *Ent. faecalis* disinfection with KPS dosages(0.1, 0.7, 1.5 and 2 mMol/l)

<i>Ent. faecalis</i> Density	Contact time(h)					
	1	2	3	4	5	6
1000 CFU/ml	0	0	0	0	0	0
Solar UV intensity($\mu\text{W}/\text{Cm}^2$)	3837.5	5157.5	6263.3	6000	4926.7	3770

Table 5) The effect of contact time on *Ent. faecalis* disinfection with KPS dosages (0.1, 0.7, 1.5 and 2 mMol/l)

<i>Ent. faecalis</i> Density	Contact time(h)					
	1	2	3	4	5	6
1500 CFU/ml	0	0	0	0	0	0
Solar UV intensity($\mu\text{W}/\text{Cm}^2$)	4115	5176	4932.5	5102.5	5087.5	4452.5

Discussion

The results of this reaserch demostarted that the application of single SODIS and KPS associated with SODIS led to decreasing of *Ent. faecalis* contamination risks in which the KPS associated with SODIS efficiency is higher than the single SODIS. Therefore, based on WHO classification, the KPS associated with SODIS and single SODIS led to providing of the safe and low risk drinking water, respectively (18). Amin et al. (2014) was reported that safe drinking water providing with SODIS need for 6 h of contact time (12) which higher than this research study in which complet disinfection of *Ent. faecalis* was performed within 2 h of contact time. Variation in required disinfection time may relevant to geographical latitudes of the pilot fields as an efficient variable which provides different intensity of solar irradiation and UV fluency (4). Although, in the present study the recorded UV radiation (max=6000 $\mu\text{W}/\text{Cm}^2$) is lower than the previous studies (38.4 W/Cm^2) (4) but experimental trials revealed that these range of UV irradiation (3770-6000 $\mu\text{W}/\text{Cm}^2$) is acceptable for SODIS operation. Based on these UV fluencies, it can be concluded that several cities in Iran have appropriate status for using of common sun irradiation for assured water providing that can be applied in field for water-borne diseases control and health promotion. Contact time is another operational parameter which should be considered in SODIS application. As shown in findings of the present study, completely disinfection with single SODIS need for 2 h of contact time for 1000 CFU/ml and 1500 CFU/ml of bacterial density, hence, 1 h of contact time can provide low risk water with 10 CFU/ml and 20 CFU/ml of residual bacteria for 1000 CFU/ml and 1500 CFU/ml of initial density of bacteria, respectively. Alikhani et al. (2011) was reported similar results that increasing of contact time led to elevation of *E.Coli* disinfection performance. Therefore,

increasing of contact time from 15 min to 2 h led to increasing of *E.Coli* removal efficiency from 22.4 to 72.4%. This phenomenon related to formation of high quantity of free radicals in high contact time (19). Ghanizadeh et al. (2015) was reported that increasing of contact time can lead to inducing of UVA spectrum which influence disinfection performance (2). Bacterial density is the most important parameter which its influences on disinfection efficacy should be considered. As demonstrated in the single SODIS part of the present study, increasing of *Ent. faecalis* concentration from 1000 to 1500 CFU/ml directed to increasing of required contact time up to 2 h. These findings comply with Alikhani et al. results which reported that increasing of *E.coli* density from 3×10^5 to 1.4×10^{12} CFU/l led to decreasing of bacterial removal rate from 90.2 to 65.5 % (19). Although, tremendous interventional-epidemiological researches had reported the performance of solar disinfection for water-transmitted diarrhea, but this process has several limitations, including water turbidity which finited to low muddy waters (<30NTU) (20). Dunlop et al. (2011) reported that elevation of water turbidity up to 50 NTU in *E. coli* water disinfection with TiO_2 associated with SODIS directed to elevation of needed contact time for entire disinfection (21). Peter et al. (2003) was reported the reducing effects of water turbidity on SODIS performance with influencing of UV penetration (22). Ghanizadeh et al. (2015) was reported that *E.coli* water disinfection with single SODIS and KPS associated with SODIS was not influenced with water turbidity up to 150 NTU (2). The results of the preent research study implied that *Ent. faecalis* disinfection was not influenced via water turbidity up to 200 NTU. Different required contact time for complete disinfection may attribute to bacteria cell wall charachteristics and its resistancy to undesirable environmental status. High performance of KPS associated with SODIS system up to 200 NTU of water turbidity can be discussed by Harding (2012) finding which

reported that the UVA lengths inactivate bacteria by stimulating dissolved organic carbon (DOC) in water, which in turn leads to the occurrence of reactive oxygen species (ROS); so, it can be claimed that turbidity origins may lead to entrance of organic compounds in water body and enhancing disinfection process (5).

McGuigan *et al.* (1998) was reported that entire inactivation of high concentrations of the faecal index organism, *Escherichia coli*, in highly muddy water (approximately 200 NTU) within 7 h which complies with the present study findings (23). According to the findings of the present study it can be claimed that KPS has significant effects on water disinfection rate. Saïen *et al.* (2011) was reported that association of KPS with UV is more efficiently than H_2O_2 and UV integrated system (24). This phenomenon can be discussed based on KPS dissociation in the water systems which directs to simultaneously formation of high performance radicals (sulfate and hydroxyl) which influence decomposition and elimination of several pollutants, notably the organic contaminants from aqueous solutions. Since, the predominant composition of bacterial cell wall are organic compounds, these radicals injured these compounds and lead to the disfunction of bacterial cell wall roles by the oxidation and bacterial injuries. High performance of KPS in water disinfection is related to the formation of very strong $SO_4^{\cdot-}$ radical ($E_0=2.5-3.1$ v) which has higher oxidizing ability than OH^{\cdot} radicals. Hence, activation of KPS needs lower energy than H_2O_2 , on the other hand, KPS is a soluble catalyst which no need for advanced separation system from water (25). According to high potential of KPS for water disinfection and no limitations from the point of the dissolved solids concentration as disinfection by-products, it can be concluded that KPS can be used as a safe enhancer for water purification in the remote areas, emergency situations and short time applications.

Conclusion

High incidence of fatality especially in developing countries relevant to bacterial polluted water implies that elimination of water transmitted bacteria for safe potable water supply via inexpensive and simple disinfection procedures is crucial and must be considered as an important point in health policies. Although, SODIS is an environmentally promising technique for water decontamination and recognized by the UNICEF, but have some drawbacks relatively long disinfection time (approximately 6 h) which need for the effective modification efforts. KPS is an appropriate, low-cost and effective water soluble catalyst which leads to simultaneously formation of effective free radicals ($SO_4^{\cdot-}$ and OH^{\cdot}) with UV spectrum and temperature. Since, the application of this compound was not direct to depletion of water quality from point of total dissolved solid concentration; so, the assessment of SODIS by KPS can be applied as a novel procedure for faecal contamination and water borne diarrhoea prevention in which disinfection time was significantly less (maximum 1 h), when SODIS was used alone. KPS has significantly enhancing effect on SODIS efficiency (over than to 2 fold) that can noticeably support societies who need for water disinfection in household and individual scale; so, it can be used as an available and smooth point-of-use procedure for providing of the safe drinking water in remote area, low-income and emergency status. Since, *Ent. faecalis* regrowth was not occurred in disinfected water via KPS associated system, it can be claimed that this process can be used as an assured technique for safe drinking water supply in field status. Based on these results the KPS dosages (0.1, 0.7, 1.5 and 2 mMol/l) has not effect on disinfection performance and completely disinfection was performed within 1 h. So, it can be concluded that KPS process can lead to accelerated disinfection which can be

occurred within 1 h contact time and 0.1 mMol/l of KPS. Elevation of water turbidity up to 200 NTU had not influenced the disinfection efficiency and in both processes completely disinfection was performed.

Footnotes

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Conflict of Interest:

The authors declared no conflict of interest.

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