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Original Research

Disinfection of UASB-Treated Municipal Wastewater by H_2O_2, UV, Ozone, PAA, H_2O_2/Sunlight, and Advanced Oxidation Processes: Regrowth Potential of Pathogens

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Abstract

Disinfection of anaerobically treated municipal wastewater was done using ozone, UV, H_2O_2, peracetic acid, and advanced oxidation processes (O_3/H_2O_2, O_3/UV, H_2O_2/UV, H_2O_2/sunlight). Ozone, UV irradiation, and H_2O_2 eliminated 99% of pathogens (total coliform, fecal coliform, and *E. coli*) at ozonation time of 20 min at the rate of 1000 mg/hr; UV irradiation time of 11 min and H_2O_2 dose of 336 mg/L. The use of combined systems (H_2O_2/sunlight) is especially promising due to the synergistic effect and cost efffectiveness. Regrowth of pathogens increased with increase in time and temperature (from 15 to 35ºC). The presence of nutrients and fluorescent light enhanced the rate of reactivation of pathogens.

Keywords: disinfection, UASB-treated wastewater, advanced oxidation processes, H_2O_2/sunlight, regrowth

Introduction

Disinfection of anaerobically treated wastewater is done to remove pathogens and bring its quality to irrigation standards [1]. The advanced oxidation processes (AOPs) are giving promising results for the disinfection of a variety of wastewaters [2-5]. AOPs are preferred over conventional treatment techniques because of several distinct advantages [6-9]. AOPs are based on the production of secondary radicals (OH^-), which are considered as the most reactive oxidizing agents in water treatment and are being used for the disinfection of microorganisms [10].

Ozone is a powerful disinfectant of pathogenic organisms and viruses. It destroys the nucleic acid compounds such as purine and pyrimidine [11, 12]. The suspended solids and dissolved organic matter can directly reduce the disinfection capacity of ozone. Although ozone and UV provide a high level of disinfection, regrowth and recovery of injured microflora can take place in treated water [13-15]. Hydrogen peroxide is very effective in killing microorganisms and provides long-term disinfection. Hydrogen peroxide breaks down into oxygen and water when it reacts with wastewater [16]. Disinfection by UV radiation is a safe technique that has no toxic effects. Through UV a high level of disinfection can be obtained in a short period of time and at a lower cost than chemical treatments [2, 17]. However, disinfection by UV also depends on the quality of wastewater to be treated. The suspended solids and surface fats, oils, and colored compounds reduce the intensity of radiation received by microorganisms [18, 19].

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The components of solar spectrum responsible for the inactivation of different microorganisms include UV-B (290-320 nm), UV-A (320-400 nm), and blue-to-green visible light (400-550 nm). Disinfection by sunlight is influenced by factors including oxygen content of water, pH, temperature, and turbidity [20]. Peracetic acid is reported to be a strong oxidizing agent and is effective against a wide range of microorganisms, including bacteria, virus, fungi, and spores [21]. PAA decomposes in water-forming acectic acid, hydrogen peroxide, and oxygen, which undergo decomposition to yield hydroxyl radicals that disintegrate the cell wall, inactivate the chemosomatic function of the cell membrane, and impair the functions of enzymes [22]. The amount of hydrogen peroxide used with UV is reported to be higher than that used with the ozone application [23]. The combination of O_3/UV is effective in disinfecting the microorganisms as ozone lowers the turbidity and color of wastewater, thereby enhancing the efficiency of UV irradiation [24].

In the present study the effective disinfection of pathogens was evaluated using ozone, H_2O_2, UV irradiation, PAA, and AOPs (sunlight/H_2O_2, O_3/H_2O_2, O_3/UV, and H_2O_2/UV). The regrowth potential of pathogens also was determined in disinfected wastewater for reuse purposes.

Materials and Methods

Wastewater Characteristics

Wastewater used in this study was obtained from a municipal wastewater drain carrying the effluent of residential and commercial areas including plazas, hotels, bakeries, and restaurants. Random samples were taken and prepared by integrating the water samples across the drain at different depths in order to avoid bias. The influent and effluent of UASB reactors (seeded with cow dung and activated sludge of a dairy industry treatment plant) was characterized for bacterial quality in terms of total coliform, fecal coliform, and *E. coli* in accordance with standard methods for the examination of water and wastewater [25]. The standard multiple-tube fermentation technique was applied for the measurement of microbiological parameters such as total coliform, fecal coliform (9221 B, C), and *E. coli* (9260 F). Samples were incubated according to the required conditions and were identified by specific confirmation tests. To ensure data quality five replications were carried out for each experimental test run. The characteristics of wastewater are presented in Table 1.

Regrowth was monitored in wastewater samples disinfected with (UV irradiation time of 15 min, ozonation time of 25 min, and H_2O_2 dose of 373 mg/L) and samples exposed to UV irradiation for inadequate time (3 and 7 minutes) for various time periods i.e. 1, 3, 7, 14, and 21 days. The effect of temperature of 15 and 35ºC, the presence of fluorescent light, and the presence of nutrients also was studied on the re-growth of pathogens in sterilized glass bottles.

Table 1. Characteristics of sewage wastewater and UASB-treated effluent.

Parameter	Unit	Sewage	UASB Effluent
pH	-	7.39[a] (0.27)	7.299 (0.16)
COD	mg/l	474.39[a] (36.51)	91.86 (8.62)
BOD_5	mg/l	245.9[b] (28.15)	43.29 (6.42)
Conductivity	mS	1.39[a] (0.51)	1.016 (0.44)
Turbidity	FTU	69.38[a] (7.95)	11.108 (0.95)
TSS	mg/l	379[a] (38.29)	90.44 (6.82)
Chlorides	mg/l	69.48[b] (7.75)	26.84 (3.78)
Color	absorbance	0.0612[b] (0.02)	0.022 (0.01)

[a, b] is average of ten and five values respectively and values in parenthesis show standard deviation

Experimental

Ozonation was performed in a bubble column reactor made of plexiglas. The internal diameter and height of the reactor was 3.5 cm and 35.5 cm, respectively. Ozone at the rate of 100 mg/hr was bubbled through the reactor by means of a diffuser at the bottom of the reactor by using an ozone generator (Enalay HGOZ 1000). The ozonation time varied from 5 to 25 min for solo experiments and for O_3/H_2O_2, 112 mg/L of H_2O_2 was added to the sample and then ozonation time was varied from 3 to 10 min. Ozone-treated effluent was sampled at desired intervals for the determination of the pathogens.

A hydrogen peroxide reactor consisted of a graduated Pyrex glass cylinder and a magnetic stirring set up. Analytical-grade hydrogen peroxide (35% w/w) from Merck was used. H_2O_2 dose varied from 112-373 mg/L. PAA was prepared in different concentrations (1, 5, and 15%) according to standard methods [25]. For each concentration of PAA, a fixed dose of 1ml/L was used.

UV irradiation alone and in combination (H_2O_2/UV) was carried out in a cylindrical reactor with an internal diameter of 3.5 cm and volume of 330 ml. The reactor was wrapped with aluminum foil in order to enhance the absorbance of UV irradiation. A low-pressure mercury UV lamp (Penray 3SC9 Upland, CA USA) with radiation intensity of 5 mW/cm^2 (at the surface of the lamp) and wavelength of 254 nm was used. The UV lamp was placed at the center of the reactor to ensure uniform distribution of UV irradiation and no lamp cooling was provided. All the experiments were performed in batch mode and at ambient temperature. UV irradiation time was varied from 3 to 15 min for UV solo experiment and for H_2O_2/UV. 112 mg/L of H_2O_2 was added to the sample and then UV irradiation time was varied from 10 to 60 sec.

Disinfection by solar radiation was undertaken using a rectangular (40×5×5 cm) reactor made of plexiglas. For H_2O_2/sunlight, 112 mg/L of H_2O_2 was added to the sample and then sunlight exposure was varied from 20 to 80 min.

The experiments were conducted under an average visible light intensity of 765 lux. All the experiments were carried out in batch mode, and each batch consisted of 300 ml UASB effluent.

Results and Discussion

Application of Ozone (O_3)

Ozone destroys pathogens either by oxidizing lipids and/or damaging the nucleic acid compounds like purine and pyrimidine [26]. Fig. 1 shows the efficacy of ozone for the removal of pathogens. The results demonstrated that the reduction in the population of pathogens (total coliform, fecal coliform, *E. coli*) was predominately dependent on ozone dose and exposure time [27, 28]. More than 99.9% disinfection was observed at 25 minutes ozonation time at the rate of 1000 mg/hr. The results show that some ozone was used up for color removal and/or to degrade dissolved organic matter [13, 29, 30], and *E. coli* were easy prey to ozone as compared to fecal coliform and total coliform. The quality of wastewater, type and number of microorganisms also controlled the disinfection performance of ozone.

Application of UV Irradiation

UV irradiation inactivates the pathogen by penetrating into the cell and altering the DNA and RNA. Fig. 2 shows that disinfection efficiency of UV irradiation has increased with increase in UV contact time. A higher UV dose ultimately ruptured the cell membrane and caused its death [14]. Pathogen removal of 99.9% was achieved at UV irradiation exposure time of 15 minutes, which may be attributed to higher TSS contents of wastewater of the reactor because the presence of TSS reduces the penetration of UV radiation in wastewater and, as a result, the intensity of radiation received by microbes is substantially lowered [18, 31]. The disinfection efficacy of UV irradiation varied with pathogen type, and *E. coli* proved less resistant to UV irradiation as compared to fecal coliform and total coliform [17, 26].

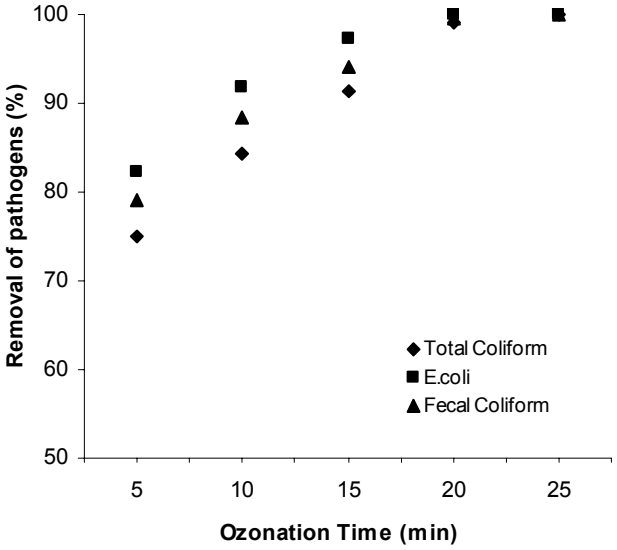

Fig. 1. The influence of ozonation time on its pathogen removal efficiency (%).

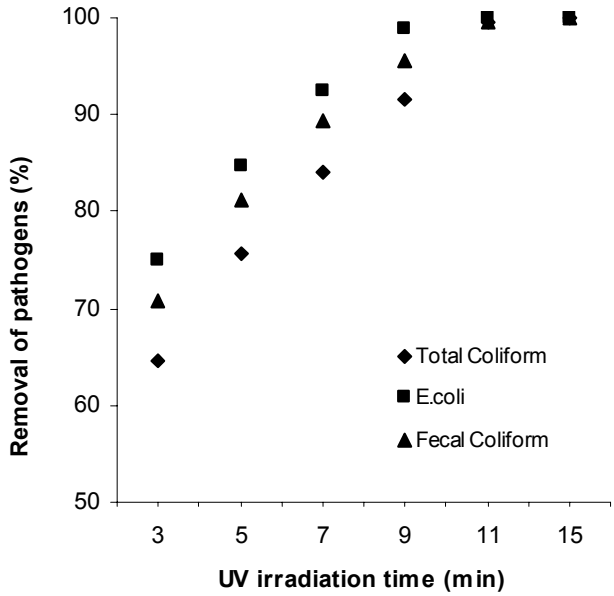

Fig. 2. The pathogen removal efficiency (%) of UV irradiation.

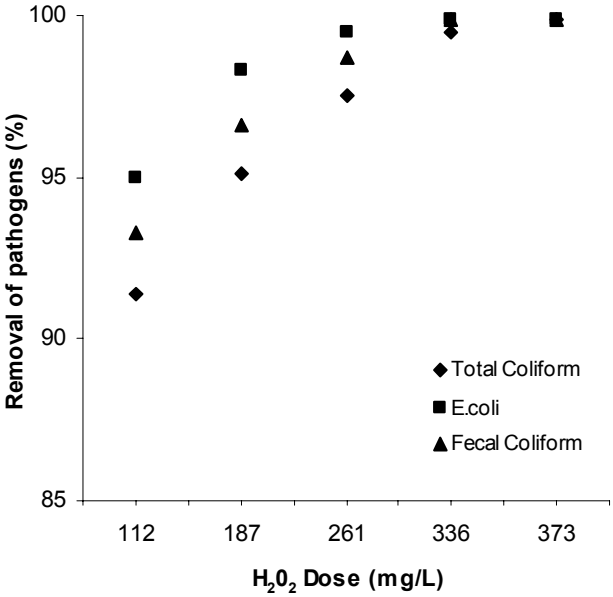

Fig. 3. The influence of H_2O_2 dose on its inactivation efficiency (%).

Application of Hydrogen Peroxide (H_2O_2)

The effect of H_2O_2 on the removal of pathogens is shown in Fig. 3. The removal of pathogens increased with an increase in the dose of H_2O_2 [3, 32]. However, a dose of 336 mg/L was found to be optimal, resulting in 97-99.9% removal of the pathogens from wastewater.

Table 2. The performance of peracetic acid for the removal of pathogens.

Pathogens	Percentage removal of pathogens at different concentrations (%) of peracetic acid		
	1	5	15
E. coli	98	99.5	99.9
Fecal coliform	97	99.0	99.9
Total coliform	96	98	99.9

The antimicrobial action of H_2O_2 owes to its oxidative properties in molecular form and generation of powerful species such as nascent oxygen, superoxide radicals, and hydroxyl radicals in wastewater. These highly reactive oxidants cause permanent damage to host cell components like enzymes, membrane ingredients and DNA, leading to alterations in cell physiology and delays in growth [16].

Application of Peracetic Acid (PAA)

The disinfection efficiency of PAA is found to be dependent on its concentration, and removal of the pathogens was maximum (99.9%) at 15% concentration at a constant dose of 1 ml/l (Table 2) as reported by other research workers [32, 33]. The application of PAA for disinfection of wastewater is advantageous due to its non-toxic effect, non-dependence on pH, short contact time, and ability to disinfect wastewater of higher suspended solid contents [21-22].

Application of Combined Systems (H_2O_2/Sunlight, O_3/H_2O_2, H_2O_2/UV, and O_3/UV)

Pathogen removal of more than 99.9% was observed at sunlight exposure time of 80 min. and H_2O_2 dose of 112 mg/L (Fig. 4). The application of sunlight with H_2O_2 significantly enhanced the rate of pathogen inactivation due to photolysis of hydrogen peroxide. Solar irradiation in the presence of 10 mg/L peroxide showed good potential of disinfection and led to 99.9% inactivation of pathogens. Moreover, the inactivation was found to be dependent on the type of microorganism as *E. coli* were less resistant to solar treatment than fecal coliform [34-37]. The use of peroxone provided 99.9% elimination of pathogens at 10 minute ozonation and H_2O_2 doses of 112 mg/L (Fig. 5). The ozonation time in the case of the O_3/H_2O_2 system was significantly reduced as compared to time required by ozone alone. The addition of H_2O_2 enhanced ozone decomposition and mass transfer, resulting in a higher yield of hydroxyl radicals and ozone reaction rate [38].

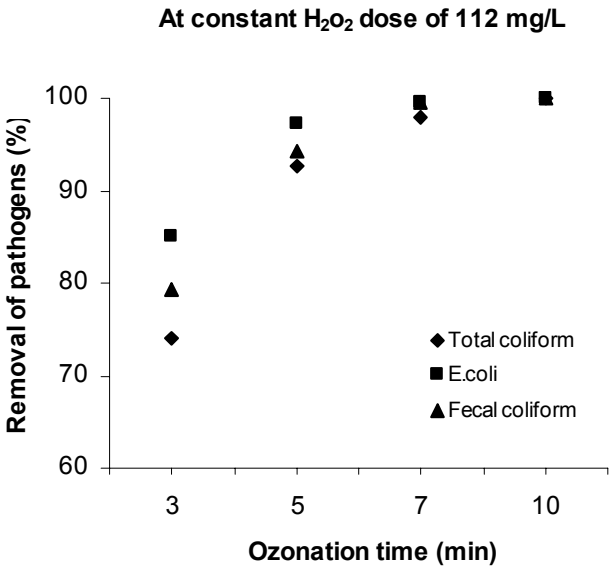

Fig. 5. The performance (%) of the O_3/H_2O_2 system for the removal of pathogens.

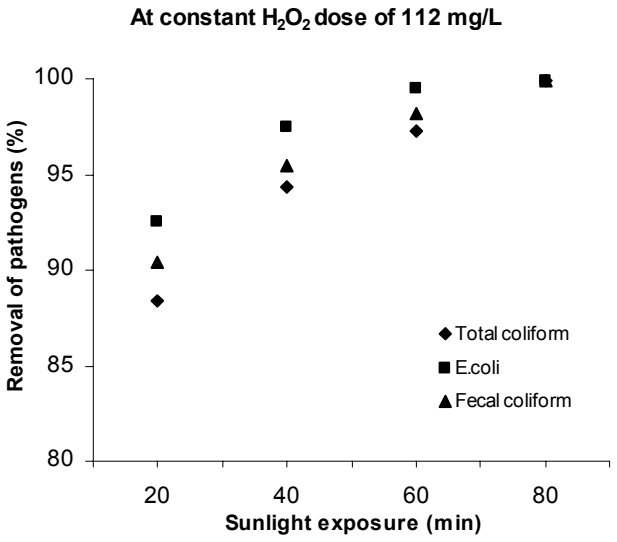

Fig. 4. The inactivation efficiency (%) of the H_2O_2/sunlight system.

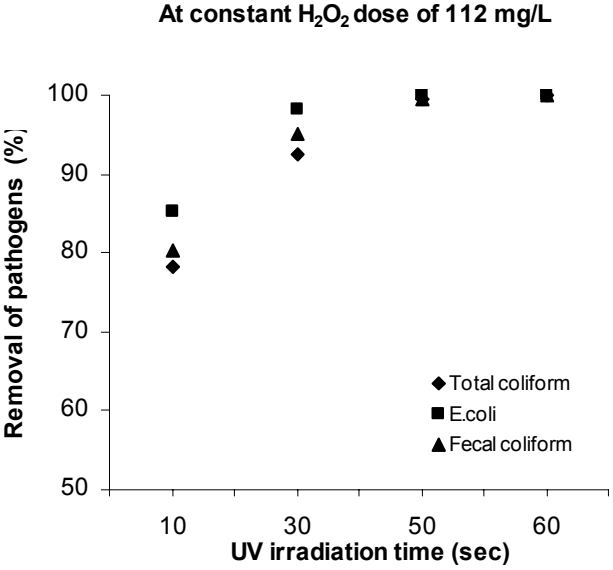

Fig. 6. The inactivation efficacy (%) of the H_2O_2/UV system.

In the case of the H_2O_2/UV process, 99.9% inactivation of pathogens was achieved with UV irradiation time of 60 sec and H_2O_2 dose of 112 mg/L (Fig. 6). The process resulted in a 87-93% reduction in UV irradiation time as compared to time required by UV alone, which may be attributed to the fact that an adequate dose of H_2O_2 absorbed UV radiation and generated hydroxyl radicals that further underwent a decomposition and formation cycle [39-41]. An adequate dose of H_2O_2 is important because its higher dose hampers the generation of hydroxyl radicals due to the scavenging effect [6, 42]. Fig. 7 shows 99.9% reduction in the population of pathogens with the O_3/UV at 10 min ozonation and UV irradiation time of 300 sec. The higher efficiency of the O_3/UV system is due to the fact that UV energy activates ozone molecules and enhances the formation of hydroxyl radicals [24, 43]. Moreover, ozone lowers the turbidity and color of wastewater, thereby enhancing the efficiency of UV irradiation [10, 29].

Re-Growth Potential of Pathogens

Regrowth is found to be dependent on temperature, nutrient content, and residual dose of disinfectant [13, 44]. Maximum disinfectant dose or adequate exposure time (ozonation time of 25 minutes, UV irradiation of 15 minutes, and H_2O_2 dose of 373 mg/L) completely eliminated the pathogens and almost no regrowth was observed at temperature of 15ºC and 35ºC, in florescent light, in darkness, and in the presence of nutrients [45]. This could be due to complete destruction of cell or damage caused to DNA, which prevented DNA from replicating during 21 days [6, 45]. There is an inverse relationship between the applied UV dose and the ability of the microorganism to repair its DNA [46, 47]. Therefore, a higher disinfectant dose is a critical factor for disrupting the repair mechanism of microorganisms and avoiding regrowth [48].

Regrowth of pathogens at 15 and 35ºC in wastewater disinfected with UV irradiation for three minutes is shown in Fig. 8. It is evident from the results that temperature enhanced the rate of regrowth of pathogens, and the maximum regrowth of total coliforms was 68 and 81% after 21 days at 15ºC and 35ºC, respectively. A comparison of regrowth in florescent light, in darkness, and in the presence of nutrients is shown in Fig. 9. The rate of regrowth of total coliform in wastewater samples stored in fluorescent light was slightly higher as compared to samples stored in darkness, indicating that fluorescent light enhanced the repair of bacteria. Faster regrowth occurred due to photoreactivation and less regrowth due to dark repair after exposure to low UV dose [34, 49, 50]. The photoreactivation process depends on the presence of light in wavelength range of 300 to 500 nm to complete the repair process. However, in the presence of nutrients the rate of regrowth was substantially higher (up to 80%) in UV-disinfected wastewater.

The effect of temperature on the regrowth of pathogens in effluent disinfected with a longer UV irradi-

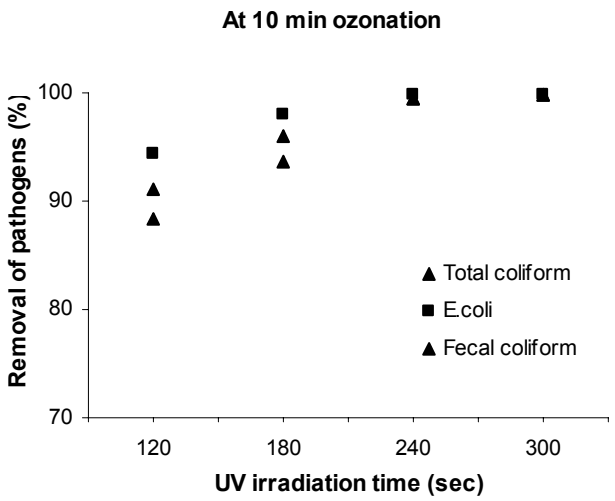

Fig. 7. The removal of pathogens by the O_3/UV system in effluents.

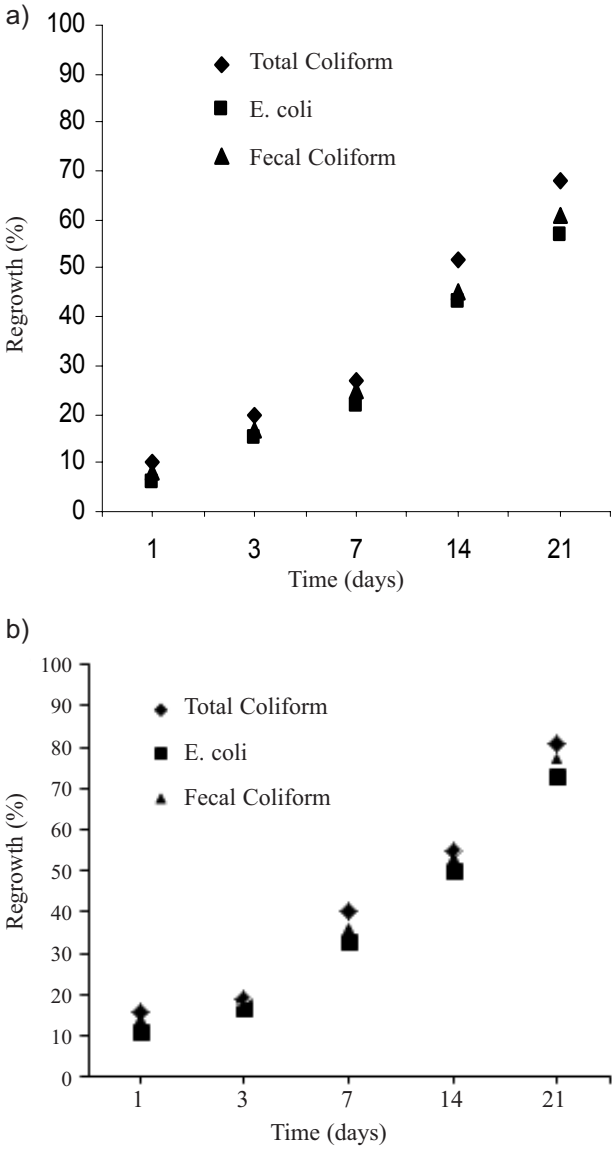

Fig. 8. Regrowth (%) of pathogens in UASB effluent disinfected with UV irradiation for 3 min at: (a) 15ºC and (b) 35ºC.

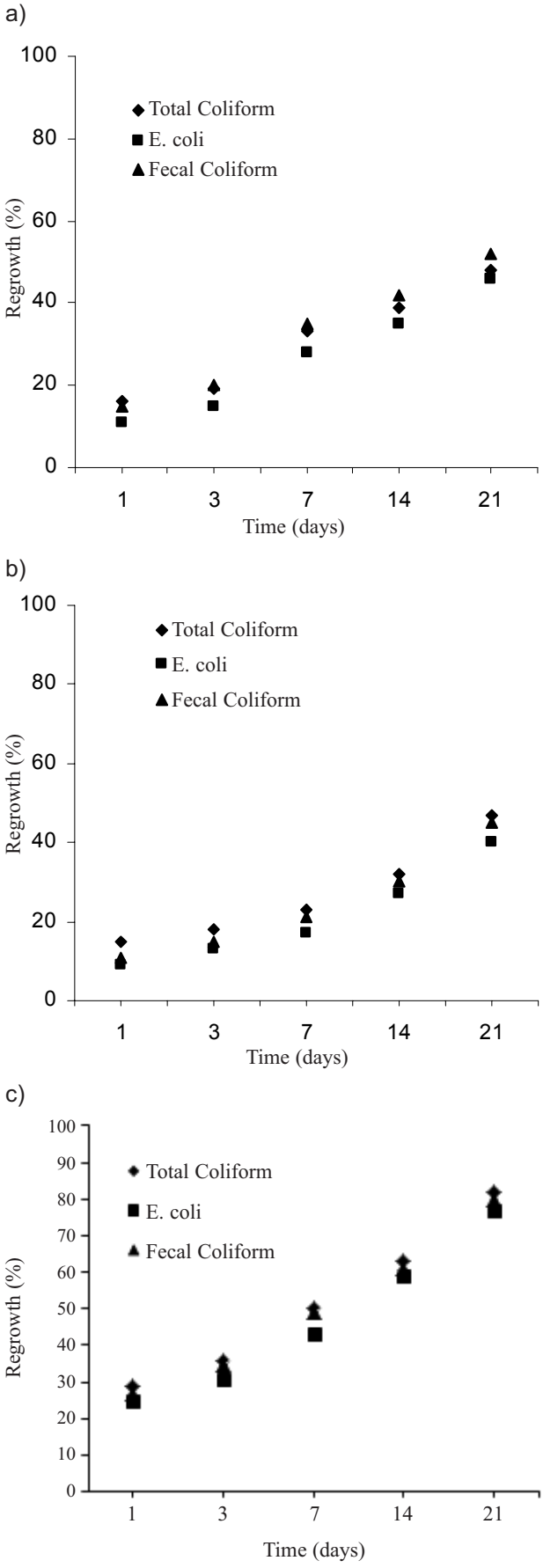

Fig. 9. Regrowth (%) of pathogens in UASB effluent disinfected with UV irradiation for 3 minutes in the (a) presence of light, (b) absence of light, and (c) presence of nutrients.

ation time (7 min) is shown in Fig. 10. Reactivation was not observed up to 2 days, beyond which rapid regrowth occurred in both wastewater samples stored at 15ºC and 35ºC temperatures, which may be due to the reactivation of UV-resistant species, which led to a high post-irradiation recovery of pathogens [51]. Maximum regrowth of total coliform was 55 and 81% at 15ºC and 35ºC, respectively. No regrowth occurred on the first day in the presence and absence of light after exposure to longer UV contact time of 7 min, which shows that sufficient contact time of UV irradiation slowed the process of reactivation pathogens and less regrowth occurred in the absence of light (Fig. 11). However, in the presence of nutrients there was significant regrowth of pathogens even on the first day, which reached 80% after 21 days as the availability of nutrients provided a fertile substratum for the microorganisms to repair their DNA at a favorable temperature [52, 53].

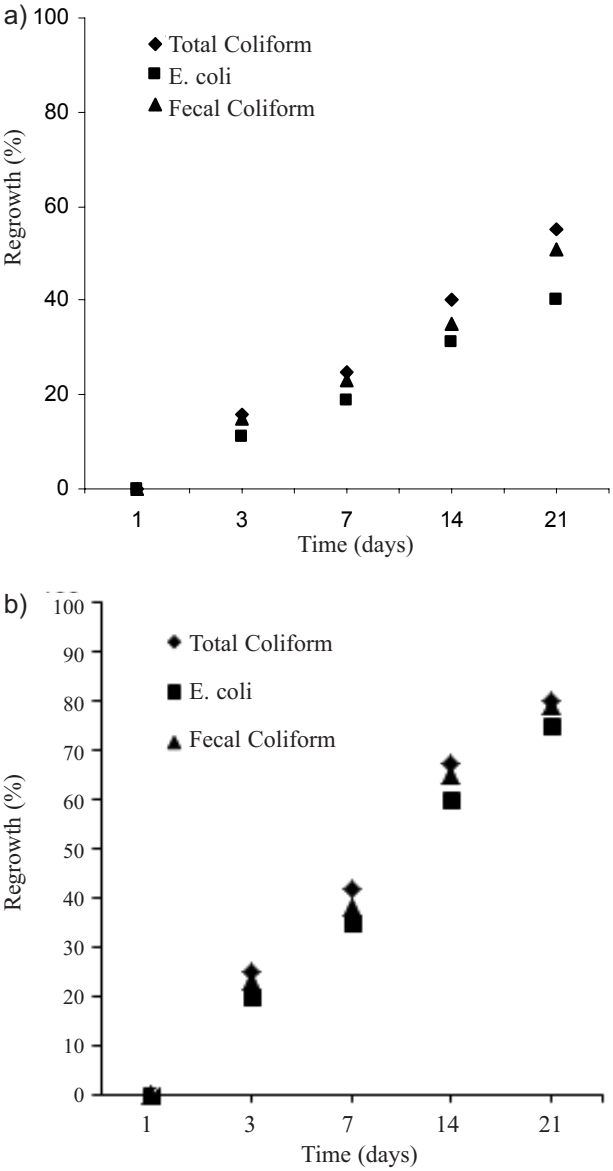

Fig. 10. Regrowth (%) of pathogens in UASB effluent disinfected with UV irradiation for 7 minutes at (a) 15ºC and (b) 35ºC.

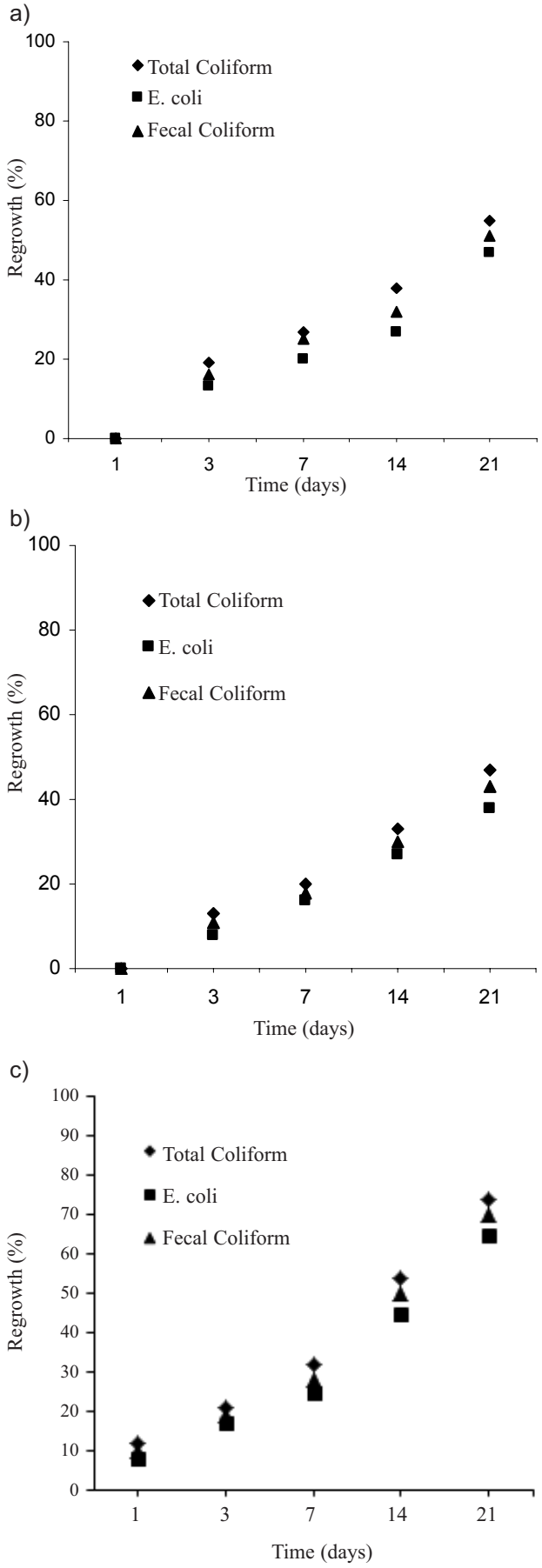

Fig. 11. Regrowth (%) of pathogens in UASB effluent disinfected with UV irradiation for 7 minutes in the (a) presence of light, (b) absence of light, and (c) presence of nutrients at 35ºC.

Conclusions

A high level of disinfection can be achieved by the use of ozone, UV, H_2O_2, and PAA. The combined systems (H_2O_2/sunlight, O_3/UV, H_2O_2/UV, and O_3/H_2O_2) showed spectacular disinfection efficiency at short contact time and less dose. The use of solar-assisted photolysis of hydrogen peroxide (H_2O_2/sunlight) is an attractive option for areas experiencing 8 hours of sunlight.

Maximum disinfectant dose or sufficient exposure to disinfectant (ozone, UV, and H_2O_2) eliminated the pathogens and almost no regrowth was observed at temperature of 15ºC and 35ºC, in florescent light, darkness, and in the presence of nutrients up to 21 days. This can be attributed to the complete destruction of cell or damage caused to DNA, which prevented DNA replication. However, underdozed samples or wastewater samples exposed for an inadequate time period to UV irradiation showed regrowth, which was a function of temperature, nutrient content, and light.

Fluorescent light increased the rate of reactivation due to photoreactivation and less regrowth occurred due to dark repair up to 21 days. Fast and higher regrowth was observed in the presence of nutrients as the availability of nutrients facilitated the microorganisms in repairing their DNA at favorable temperature.

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