Original article

The effect of temperature on peracetic acid efficiency in fungal elimination in reused dialyzer

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Abstract:

Introduction: *Peracetic acid (PAA) decomposition upon dialyzer reprocessing varies with elevated temperature.* ***Purpose:*** *The current objective is to investigate the antibacterial activity against Candida albicans and Aspergillus niger of 0.16% PAA in dialyzers at elevated temperature 35-50 Celsius (°C) after incubations for 0-96 hour (h).* ***Material and methods:*** *Twenty dialyzers were randomly assigned to 1.1) C. albicans-inoculated control group of dialyzers, 1.2) C. albicans-inoculated sample group of dialyzers with PAA 2.1) A. niger-inoculated control group of dialyzers 2.2) A. niger-inoculated sample group of dialyzers with PAA. Then a residual concentration of PAA in sample group of dialyzers were quantified with peracetic acid test strip.* ***Result:*** *At 35, 40, and 45°C, the sample group number 1.2) and 2.2) showed that PAA started decomposing at 48 h. The amount of PAA residues at 96 h decreased slightly from 2,000 parts per million (ppm) to 1,500 ppm at 35°C, and decreased dramatically to 1,000 ppm at 40 and 45°C. The highest rate of PAA degradation was found at 50°C with a rapid decline from 1,500 ppm at 24 h to 800 ppm at 96 h. Although PAA decay is associated with rising temperatures, both sample groups still showed strong bioactivity against both fungi, suggesting that PAA concentrations remain sufficient for disinfection.* ***Conclusion:*** *The concentration of PAA presented in dialyzers for use at high temperature must be at a minimum level of 2,000 ppm and 800 ppm before and after reprocessing procedure, respectively.*

Keywords: ● *Dialyzer reprocessing* ● *Dialyzer reuse* ● *Peracetic acid* ● *Temperature*

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นิพนธ์ต้นฉบับ

ผลของอุณหภูมิต่อประสิทธิภาพของกรดเปอราซิติกในการกำจัดเชื้อรา ในตัวกรองเลือดที่ใช้ซ้ำ

สุวดี โพธิ์วิจิตร[1] นันทนา ชปิลเลส[2] สุนทรี เพิ่มพูลสวัสดิ์[3] ธีราวุฒิ ภู่สันติสัมพันธ์[4] วรวิทย์ รัตนวงษ์[1] และ วีระศักดิ์ อัศววงศ์อารยะ[1]

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บทคัดย่อ

บทนำ *การสลายตัวของกรดเปอราซิติกในกระบวนการนำตัวกรองเลือดกลับมาใช้ซ้ำแปรผันตามระดับอุณหภูมิที่สูงขึ้น* ***วัตถุประสงค์*** *เพื่อศึกษาประสิทธิภาพของกรดเปอราซิติก 0.16% ในตัวกรองเลือดที่ใช้ซ้ำต่อการกำจัดเชื้อCandida albicans และ Aspergillus niger ที่อุณหภูมิ 35 ถึง 50 องศาเซลเซียส (°ซ.) เวลา 0 ถึง 96 ชั่วโมง (ชม.)* ***วัสดุและวิธีการ*** *แบ่งตัวกรองเลือดจำนวน 20 ตัวแบบสุ่มเป็น 1.1) กลุ่มควบคุมคือตัวกรองที่ปนเปื้อน C. albicans 1.2) กลุ่มทดลองคือตัวกรองที่ปนเปื้อน C. albicans ที่มีกรดเปอราซิติก 2.1) กลุ่มควบคุมคือตัวกรองที่ปนเปื้อน A. niger และ 2.2) กลุ่มทดลองคือตัวกรองที่ปนเปื้อน A. niger ที่มีกรดเปอราซิติก จากนั้นทดสอบความเข้มข้นคงเหลือของกรดเปอราซิติกในกลุ่มทดลองด้วยแผ่นทดสอบ* ***ผลการศึกษา*** *ในกลุ่มทดลอง 1.2) และ 2.2) ที่อุณหภูมิ 35, 40, และ 45°ซ. พบว่า กรดเปอราซิติกเริ่มสลายตัวที่ 48 ชม. โดยที่ 96 ชม. สลายตัวจาก 2,000 เหลือ 1,500 ppm ณ อุณหภูมิ 35°ซ. และลดลงเหลือ 1,000 ppm ที่อุณหภูมิ 40 และ 45°ซ. ตรวจพบการสลายตัวสูงสุดที่อุณหภูมิ 50°ซ. เท่ากับ 1,500 และ 800 ppm ที่ 24 และ 96 ชม. แม้สารจะสลายตัวตามอุณหภูมิที่สูงขึ้นแต่พบว่าฤทธิ์ของสารในกลุ่มทดลองยังสามารถยับยั้งการเจริญเติบโตของเชื้อราทั้งสองชนิดได้ ดังนั้นความเข้มข้นที่เหลืออยู่เพียงพอต่อการฆ่าเชื้อโรค* ***สรุป*** *ควรกำหนดความเข้มข้นของกรดเปอราซิติกที่ใช้ในตัวกรองเลือดที่ใช้ซ้ำเมื่ออุณหภูมิสูงขึ้น โดยให้มีค่าก่อนและหลังทำการอบตัวกรองอย่างน้อยที่ 2,000 ppm และ 800 ppm ตามลำดับ*

คำสำคัญ: ● *กระบวนการนำตัวกรองเลือดกลับมาใช้ซ้ำ* ● *ตัวกรองเลือดที่ใช้ซ้ำ* ● *กรดเปอราซิติก* ● *อุณหภูมิ*

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Introduction

Reused dialyzers have been used worldwide on long-term Hemodialysis (HD) due to increased biocompatibility, avoiding the risk of first-used syndrome. This reduces manufacturing costs by decreasing the amount of non-biodegradable materials. However, it is imperative to ensure that dialyzers meet reprocessing standards required by the American Association for the Advancement of Medical Instrumentation (AAMI) before administration in patients[1]. In Thailand, Reused dialyzers have also been widely used in compliance with the Thai Nephrology Nurse Society. The suggested disinfectant is peracetic acid (PAA)[2] since it is a high-level oxidizing agent for inactivation of broad-spectrum microbial pathogens that cause infectious diseases in humans[2]. Four percent PAA solution is diluted with reverse osmosis (RO) purified water to its effective concentration at 0.16% (w/v). Dialyzers treated with 0.16% PAA must be sterilized with an incubation period of 11 h for quality assurance. The capacity of each individual sterilized dialyzer can be utilized for a specified time limit of 7 days without constantly renewed PAA[2,3]. Nevertheless, previous studies demonstrated that PAA in aqueous solution is an unstable oxidizing agent. It can decompose to acetic acid, hydrogen peroxide (H_2O_2), and end-product water at high temperature with a first-order reaction[2-4]. Indeed, the highest temperature reported in Thailand at about 44 Celsius (°C) caused by global warming is an important consideration for the performance of dialyzers because it may impact the effectiveness of PAA in sterilized dialyzers as there is no temperature-controlled room for storage of dialyzers. Although it was observed that even half of 0.16% PAA in reused dialyzer performed at 45°C had strong antibacterial activity against Gram-negative *Escherichia coli* (*E. coli*) at 24 h,[5] human pathogen is not only bacteria but also includes other microorganisms. Therefore, other fungi should be additionally investigated for the current study.

Fungi are saprophytic eukaryotic organism which can be found in abundance in different environments such as tap water, hence they can survive at varying temperatures[6]. In fact, some species cause many types of human diseases e.g. candidiasis or aspergillosis by producing their bioactive compound (mycotoxins) and fungal hypha in high concentrations[7] for optimal fungal cell wall modification to protect their proliferation from any chemical sterilant[8-10]. Most investigators have found that a yeast-like fungus *Candida albicans* (*C. albicans*) is a main species that can affect candidiasis both in superficial infections and systemic infections of human[11,12]. *C. albicans* can be found in several medical devices-related nosocomial infections, for example, catheters, joints, and implant[13-15], leading to *C. albicans* involved in the systemic circulation followed by dysfunctions of adaptive immunity. Patients may have the mortality rate of 50% even when they are using medication[16]. As expected, *Aspergillus niger* (*A. niger*) is an ascomycetous filamentous fungi in which its body has black sporulation and even in adverse conditions it can cause not only aspergillosis but also otitis -or tracheobronchitis in diverse environments[17,18]. Lin *et al*., 2017 reported that a gastric cancer patient was infected with *A. niger* in bloodstream after operating with artery hepatic embolization[19]. In addition, patients with refractory glaucoma may be infected with *A. niger* through a glaucoma drainage device[20]. Most studies established that growth behaviors of yeast *C. albicans* and filamentous fungi *A. niger* were particularly recovered in water system and dialysis fluid in HD units even though water is purified by reverse osmosis[21, 22]. The virulence of these fungal strains *C. albicans* and *A. niger* is caused by their adaptation for changing water composition[6]. A presence of both fungal species in water treatment system may cause substantial contamination and eventually serious illness to patients. In this study, we emphasized the effect of temperatures in dialyzer reuse towards the effectiveness

of PAA in disinfection of fungal pathogens *C. albicans* and *A. niger* in order to research for new clinical practice guideline. It may reveal a sufficient concentration of PAA sterilant at high temperatures for protecting HD patients and fulfilling the quality of hospital care in the future.

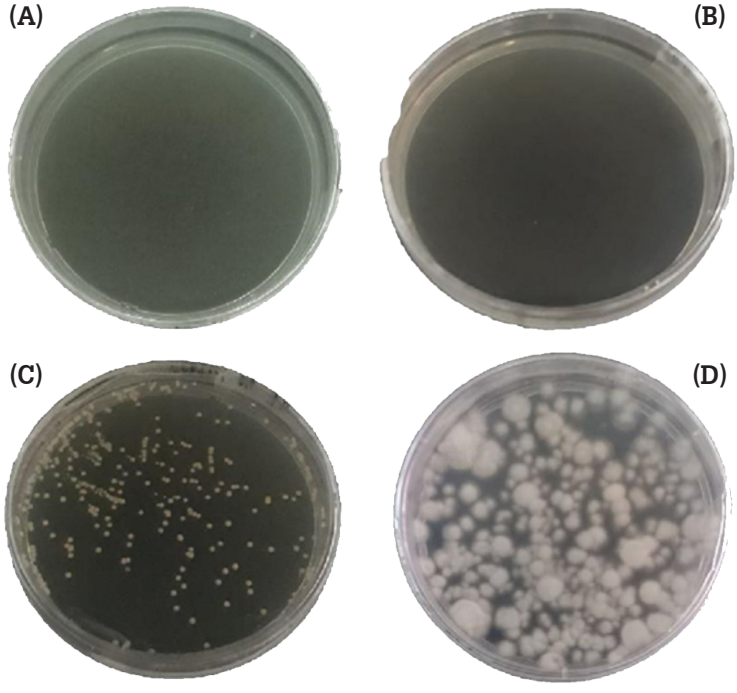
Material and Methods

Study design, dialyzer reuses, and strain

Twenty reused dialyzer were supplied by the Dialysis Unit in Nopparat Rajathani and Samutprakarn Hospitals, Thailand (with the maximum number of reuses). These dialyzers were randomly assigned to four groups (n = 5): 1.1) *C. albicans*-inoculated control group of dialyzers, 1.2) *C. albicans*-inoculated sample group of dialyzers with 0.16% PAA, 2.1) *A. niger*-inoculated control group of dialyzers, and 2.2) *A. niger*-inoculated sample group of dialyzers with 0.16% PAA. PAA was obtained from Meditop, Thailand. Strains of *C. Albicans* 10230 was obtained from the American Type Culture Collection of the United States and *A. Niger* 3254 was obtained from the Thailand Institute of Scientific and Technological Research. These fungi were then maintained by microbiological culture method on yeast malt agar (YMA) and potato dextrose agar (PDA) medium (Himedia, Laboratories, India), respectively. The dialyzer reprocessing was performed following AAMI[1].

Water purification system

The water was disinfected by passing through RO water treatment system. In Figure 1, these culture media were classified for an optimum growth of *C. albicans* and and *A. niger* in four groups: (A) control group with purified water on YMA medium; (B) control group with purified water on PDA medium; (C) sample group with *C. albicans* inoculation into water on YMA medium; and (D) sample group with *A. niger* inoculation into water on PDA medium. Fungal contamination of the purified water was tested before use, and no pathogenic yeast or filamentous fungi was found (Figure 1A and 1B). Thus the purified water cound be used to set up a research experiment in the steps described below.



Representative images in presence -or absence of *C. albicans* and *A. niger* inoculation. (**A**) without any *C. albicans* inoculation into water on YMA medium plate; (**B**) without any *A. niger* inoculation into water on PDA medium plate; (**C**) with *C. albicans* inoculation into water on YMA medium plate; (**D**) with *A. niger* inoculation into water on PDA medium plate; YMA = yeast malt agar; PDA= potato dextrose agar medium.

Figure 1 A fungal colony morphology was grown on specific nutrient media.

When these agar plates were used to grow microbes in suitable culture media at 37°C for 48 h, it revealed small colonies of *C. albicans* with white smooth spherical (Figure 1 C) and presented hyphal fringe of *A. niger* in white rough colonies (Figure 1 D). Both species developed their colonies in each growth medium, and these fungal strains were acquired from each culture medium individually.

Microbiological analyses

A procedure to study the efficiency of PAA for disinfecting *C. albicans* inside dialyzer reuse was performed with purified water system following the steps below. a) The control group of dialyzer No.1.1 was prepared by adding 10 milliliters (ml) of *C. albicans* (0.2 McFarland) suspended in 990 ml of water coupled with yeast malt broth media (YMB). After stirring well, the medium with microbial load was introduced into both dialysate and blood compartments of each reused dialyzer. b) The sample group of dialyzer No.1.2 was added 0.16% PAA (prepared from 40 ml of 4% PAA), followed by 10 ml of *C. albicans* (0.2 McFarland) with YMB media suspended in 950 ml of water. This sample suspension was infused through both dialysate and blood compartments of each reused dialyzer. c) Each dialyzer of control and sample groups was incubated at 35°C for 0, 11, 24, 48, 72, and 96 h. d) In order to quantify the C. *albicans* growth rate, 25 microliters (μL) of fungal suspension solution were obtained from the blood compartment of the dialyzers in control and sample groups following the time periods in step c. The suspension for each inoculation time of control and sample groups was spread on YMA medium plates and then cultured at 35°C for 48 h. The number of fungal colonies were counted in term of Log colony forming unit (CFU)/mL. e) The residual concentration of PAA in sample group at 35°C was measured by peracetic acid test strip (Johnson, UK) at selected time points (0-96 h). f) The effect of alternating temperatures on PAA efficiency inside reused dialyzers was observed by repeating the instruction in steps a-d and varying the incubation temperatures at 40, 45, and 50°C.

At the same time, the analyzed strain of *A. niger* inside reused dialyzers was observed by following these steps a-f. a) Each dialyzer of control group No.2.1 was prepared by directly adding 1,000 mL of water into dialysate and blood compartments. b) The dialyzer of sample group No.2.2 was prepared by adding 40 mL of 4% PAA in 960 mL of water and injecting the sterilant through both sides of dialysate and blood compartments of each dialyzer. c) Subsequently, each specimen of control and sample groups was incubated at 35°C. d) The *A. niger* filamentous was cut into 2x2 mm fragments with sterile knife and placed on the PDA medium plates, before the following tests in step e. e) To observe the *A. niger* strain growth rate of the control and sample groups of dialyzers, 5 mL of suspension from blood compartment of dialyzers were obtained from both control and sample groups at different time periods of 0, 11, 24, 48, 72, and 96 h. These suspensions were placed on the inoculated *A. niger* medium plates and then incubated at 35°C for 48 h. The fungal colony growth was recorded with digital camera. f) The amount of PAA residues in sample group No.2.2 were quantified with peracetic acid test strip at 35°C for 0, 11, 24, 48, 72, and 96 h. h) The procedure was repeatedly at different temperatures (40, 45, and 50°C) with the instruction in steps a-f.

Statistical analysis

The results were five independent experiments for statistical analysis. The *C. albicans* growth rate was represented in mean ± standard error of mean (SEM). The number of *C. albicans* colonies corresponding with varying temperatures and incubation time periods were analyzed using one-way ANOVA followed by Turkey's range test, since the p-values were less than 0.05. A branching density of *A. niger* was reported with the representative photos.

Results

The potential of PAA disinfecting dialyzer of sample group No. 1.2 (inoculated *C.albicans*) and No. 2.2 (inoculated *A. niger*) at varying time-temperature relation showed their residual concentrations in Table 1. At 35°C, the amount of PAA sterilant decreased from 2,000 to 1,500 parts per million (ppm) (25 % decomposition). The residual PAA at 40 and 45°C were also 1,500 ppm after 48 h and remained at 1,000 ppm after 96 h (50% decomposition). The PAA degradation rate increased when the temperature was raised to 50 °C. The amount of PAA decreased to 1,500 and 1000 ppm after 24 h and 48 h of incubation, respectively, whereas the lowest residues were 800 ppm at 96 h (60% decomposition). This showed that PAA decomposition depends on time-temperature conditions.

In Table 2, the number of *C. albicans* were cultured on medium plates for 48 h after collecting the suspension from dialyzers at various temperatures (35-50°C) for different length of time (0-96 h). The manifestation of *C. albicans* colony growth in control group No.1.1 (-without PAA) at 35°C had no significant difference after incubating for different time periods (11 h: 4.35 ± 1.20 log CFU/mL, 24 h: 4.51 ± 1.41 log CFU/mL, 48h: 4.72 ± 1.43log CFU/mL, 72h: 4.82 ± 1.49 log CFU/mL, and 96 h: 4.50 ± 1.47) as compared to the initial time result (4.60 ± 1.28 log CFU/mL). While there was a

Table 1 The residual efficacy of 0.16% PAA for dialyzer disinfection in sample group No.1.2 and No.2.2 under varying time-temperature conditions.

Temperature (°C)	Peracetic concentrations in ppm					
	Incubation time period					
	0 h	11 h	24 h	48 h	72 h	96 h
35	2,000	2,000	2,000	1,500	1,500	1,500
40	2,000	2,000	2,000	1,500	1,500	1,000
45	2,000	2,000	2,000	1,500	1,500	1,000
50	2,000	2,000	1,500	1,500	1,000	800

ppm = Parts per million. The residual available PAA concentrations in ppm was identified by peracetic acid test strip.

Table 2 The mean colony forming units of *C. albicans* in culture from reused dialyzers in control group No.1.1 (-without PAA) and in sample group No.1.2 (-with 0.16%PAA) at various temperatures for different time periods.

Group	Temp (°C)	Mean colony counts of *C. albicans* in Log CFU/mL					
		Incubation period of dialyzers					
		0 h	11 h	24 h	48 h	72 h	96 h
Control group 1.1 *C. Albicans* (without 0.16 % PAA)	35	4.60 ± 1.28	4.35 ± 1.20	4.51 ± 1.41	4.72 ± 1.43	4.82 ± 1.49	4.50 ± 1.47
	40	4.67 ± 1.54	4.46 ± 1.42	4.28 ± 1.28*	4.23 ± 1.00* #	4.22 ± 1.33* #	4.30 ± 1.56*
	45	4.62 ± 1.55	3.73 ± 1.27** #	3.96 ± 1.00** #	3.62 ± 0.92** #	3.45 ± 1.09** #	3.36 ± 0.99** #
	50	NG	NG	NG	NG	NG	NG
Sample group 1.2 *C. Albicans* (with 0.16 % PAA)	35	NG	NG	NG	NG	NG	NG
	40	NG	NG	NG	NG	NG	NG
	45	NG	NG	NG	NG	NG	NG
	50	NG	NG	NG	NG	NG	NG

Temp = Temperature; NG = No Growth; CFU = Colony forming units. The *C. albicans* suspension were collected at 35-50°C for 0-96 h after five sampling of dialyzers were performed by the protocol. The colony counts of *C. albicans* in control (-without PAA) and sample group (-with 0.2% PAA) were kept after incubated at 35°C for 48 h and represented in mean ± SEM; *p-value < 0.05; **p-value < 0.01 the number of colonies at different time points versus at initial time 0 h; #p-value < 0.05 the number of colonies at various temperatures versus at 35°C for the same period.

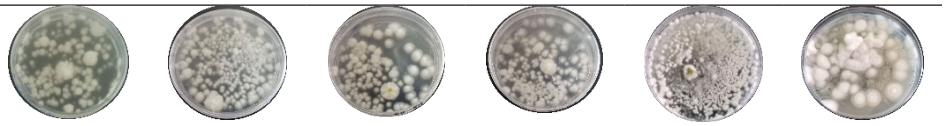
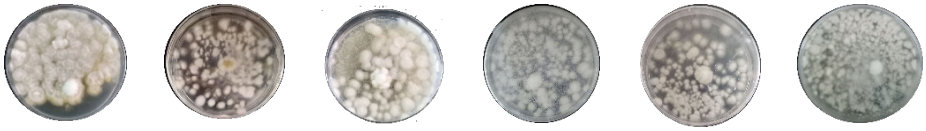
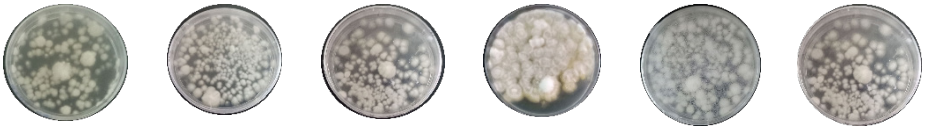
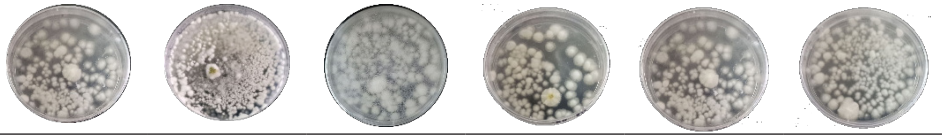
significant difference in fungal growth rates of control group at 40°C showing a decreasing prevalence from dialyzers during the incubation of 24-96 h (24h: 4.28 ± 1.28 log CFU/mL, 48h: 4.23 ± 1.00 log CFU/mL, 72h: 4.22 ± 1.33 log CFU/mL, and 96 h: 4.30 ± 1.56 log CFU/mL) as compared to that at 0 h (4.67 ± 1.54 log CFU/mL), p-value < 0.05. The colony count population from dialyzers in control group at 45°C also declined slightly as the incubation time increased (11 h: 3.73 ± 1.27 log CFU/mL, 24 h: 3.96 ± 1.00 log CFU/mL, 48 h: 3.62 ± 0.92 log CFU/mL, 72h: 3.45 ± 1.09 log CFU/mL, and 96 h: 3.36 ± 0.99 log CFU/mL) as compared with the observation at 0 h (4.62 ± 1.55 log CFU/mL), p-value < 0.01. Therefore, a significant reduction of colony counts in control group at 40°C was found in a similar manner for 48 h and 72 h with action levels 4.23 ± 1.00 vs 4.72 ± 1.43 log CFU/mL and 4.22 ± 1.33 vs 4.82 ± 1.49 log CFU/mL, respectively, as compared with the result at 35°C (p-value < 0.05 for both times). Interestingly, the density of fungal pathogens at 45°C were affected by growth restriction during time periods of 11-96 h, as compared to the result at 35°C, showing 3.73 ± 1.27 vs 4.35 ± 1.20 log CFU/mL (for 11 h), 3.96 ± 1.00 vs 4.51 ± 1.41 log CFU/mL (for 24 h), 3.62 ± 0.92 vs 4.72 ± 1.43 log CFU/mL (for 48 h), 3.45 ± 1.09 vs 4.82 ± 1.49 log CFU/mL (for 72 h), and 3.36 ± 0.99 vs 4.50 ± 1.47 log CFU/mL (for 96 h), (p-value < 0.01 for all times under consideration). Indeed, the maximum controlled temperature at 50°C had an ability to destroy *C. albicans* colony formation for all time periods. These data suggested that the disappearance of *C. albicans* strain was related to the rising temperatures of dialyzer. As the incubation time at elevated temperatures 35, 40, and 45°C had no effect on pathogens inside dialyzer at different times of 11, 24, 48, 72, and 96 h, the remaining of these fungal implied that the time treatment of this condition was not relevant to *C. albicans* growth. In contrast, administration of 0.16% PAA residing on the dialyzers in sample group No. 1.2 completely suppressed *C. albicans* proliferation for all incubation periods of each temperature tested.

In addition, we developed this model using *A. niger* as it has been previously reported to contribute to the HD system. The effect of incubation temperatures and times on PAA efficiency within individual dialyzer in control group No.2.1 (absence 0.16%PAA) and sample group No.2.2 (presence 0.16%PAA) related to the fungi disperses after we derived their suspension from sub-sampling dialyzers to culture on medium (Table 3). After adding the *A. niger* fragments into the solution of control group for 48 h, A large amount of fungal were present in white compact mycelial colonies and it was difficult to distinguish colony count data across multiple concurrent incubations at different temperatures. However, the sample group with 0.16% PAA showed strong inhibitory effect on the growth of *A. niger* within 48 h, revealing no apparent growth at all time-temperature conditions. These findings indicated that the external temperature range at 35-50°C did not affect the existence of *A. niger* after dialyzer reprocessing procedure. *A. niger* disruption occurred from the disinfection efficiency of PAA at each temperature condition.

Discussion

Microbial degradation accelerated by PAA on reprocessed dialyzers is implemented worldwide. PAA is a strong decontaminant in re-useable medical devices and produces naturally occurring products upon a spontaneous decomposition so it become less harmful in human respiratory sensitization[4,23,24]. Bond et al., 2011 reported that patients using dialyzers reprocessed with PAA had lower mortality rates as compared to a conventional single use[25]. The antimicrobial activity in dialyzers is obtained after prolonging dialyzers with 0.16% PAA for 11 h, and this activity disappears after

Table 3 The distribution of *A. niger* in culture from suspension after dialyzer administration were taken into control group No.2.1 (-without PAA) and sample group No.2.2 (-with 0.16%PAA) over a specific period as exposed to different temperatures.

Group	Temp (°C)	Distribution of *A. niger* in culture					
		Incubation period of dialyzers					
		0 h	11 h	24 h	48 h	72 h	96 h
Control group 2.1 *A. niger* (without 0.16 % PAA)	35						
	40						
	45						
	50						
Sample group 2.2 *A. niger* (with 0.16 % PAA)	35	NG	NG	NG	NG	NG	NG
	40	NG	NG	NG	NG	NG	NG
	45	NG	NG	NG	NG	NG	NG
	50	NG	NG	NG	NG	NG	NG

Temp=Temperature, NG= No Growth. Macromorphological growth of *A. niger* on the medium for 48 h of control group expressed after obtaining suspensions from the dialyzers in this approach. An overgrowth of the *A. niger* images in control group (absence 0.16% PAA) with either collected suspension from different temperatures (35-50 °C) or different periods (0-96), but in fact there were no colonies in sample group (presence 0.16%PAA) at any time-temperature in this study (not shown).

72-96 h storage periods. As patients undergo HD twice or thrice per week (the HD interval is 72 or 96 h), a maximum time limit in this study was dedicated as 96 h. Based on the prevailing of *C. albicans* and A. *niger* found in water supplies of HD unit[21,22], quantifying the existence of these pathogens upon dialyzer treatment with PAA at different temperatures (35-50 °C) and time periods (0-96 h) was clarified. The effectiveness of PAA disinfectant was illustrated by growing fungal suspensions from each dialyzer at 35°C for 48 h.

After dialyzer incubation at elevated conditions in control group No. 1.1, the medium plates at 35°C showed a number of *C. albicans*. However, the growth rate was reduced when dialyzers were incubated at higher temperature of 40°C and 45°C. The dialyzers exposed at the highest temperature of 50°C enabled total clearance of C. albicans, suggesting that the ability to reduce *C. albicans* was correlated with increasing temperatures during dialyzer exposure. The findings were consistent with Nadeem SG *et al.*, 2013 in that *C. albicans* formation developed at 37°C, whereas this decreased when the temperature exceed the optimum[26]. The results in table 2 showed that no effect of incubation time at these temperatures was observed, indicated that a decrease in reproductivity of the yeast was caused by affecting temperatures and did not depend on time periods upon

dialyzer incubation. On the contrary, the yeast colonies in sample group No. 1.2 (with 0.16% PAA) did not grow in culture at all different conditions. This summary revealed that even though PAA decayed faster at high temperatures, this remaining sterilant were still capable of eradicating yeast proliferation. After reprocessing dialyzers at 50°C for 96 h, the dialyzers were still able to remove *C. albicans* contaminants even though the PAA residues was reduced to 800 ppm from the strip test (Table 1).

We observed that *A. niger* cultured with solutions in reprocessed dialyzers formed at 48 h (after applying the same condition from *C. albicans* study). *A. niger* were fully distributed on media in control group No.2.1 throughout the entire incubation temperatures and time periods. These efforts revealed that a scorching 50°C did not involve in fungal biomass production. In contrast, none of fungal colonies in sample group No.2.2 with 0.16% PAA at all conditions were observed. The presence of 0.16% PAA in sample group No.2.2 (with *A. niger* inoculum) and sample group No.1.2 (with *C. albicans* inoculum) produced the same result as the remaining PAA of 800 ppm showed antifungal effects and damaged all pathogens. Our results displayed a continuous decrease in PAA residues, between 800 and 2,000 ppm, as temperatures and incubation time rose. This result is in accordance with the finding of Kunigk *et al*., 2001 that showed a loss of PAA caused by an increase in temperature[4]. Despite PAA decayed with varying temperatures, the concentration of 800-2,000 ppm was still remarkably effective against microbes[27-29] as H_2O_2 generated by PAA has a high diffusion rate and can go through plasma membranes to rapidly kill pathogens[30,31]. This result recommended that the maximum temperature for reused dialyzer storage is 50°C. Even if the United States Food and Drug Administration) suggested the implementation of temperature for liquid chemical sterilization about 20-25°C[27], the construction of storing cabinet for dialyzers with this temperature control range will need more investment due to high temperature in Thailand.

As these observations showed *A. niger* arising under 50°C, a dialysis center should be continuously cleaning dialyzer storage area to ensure adequate dialyzer sterilization for patients receiving HD. The use of dialyzers treated with PAA in the initial concentration less than 2,000 ppm may affect the sterilant efficiency to restrain *A. niger* invasion because the decomposition of PAA will occur and its concentration will reach below 800 ppm. To clarify an evidence of this study, we measure *A. niger* growth on medium acquired from various suspensions from dialyzers reprocessed with the desired 0.16% PAA levels of 0-800 ppm at a constant temperature of 50°C. The ratios of survival to mortality rate in *A. niger* after dialyzers exposed to PAA levels of 0, 400, 500, 600, 700, and 800 ppm y were 5:0, 5:0, 4:1, 4:1, 3:2, and 0:5, respectively. It implied that inhibition of *A. niger* growth was successful by dialyzers reprocessed with 800 ppm PAA. In the 50°C experiments, *A. niger* in control group No.2.1 were more resistant than *C. albicans* in control group No.1.1. Such finding was in accordance with a previous study showing that filamentous fungus *A. niger* displayed higher tolerance at 45-47°C than *C. albicans* yeast cells[32]. Hence, handling a filamentous fungus should be more careful to avoid contamination than handling a yeast cell.

Three clinical practice guidelines of reprocessed dialyzers are recommended. Firstly, the PAA concentration should not be less than 2,000 ppm before reprocessing. Secondly, a residual PAA should remain at least 800 ppm prior to saline rinsing. Finally, the dialyzer storage area should be regularly cleaned up to ensure no filamentous fungi from contaminated environment.

Conclusion

We concluded that PAA in dialyzer still showed high bioactivity even after reprocessing at 50°C. The residual PAA level of 800 ppm is sufficient for fungicides of both *C. albicans* and *A. niger*. The result of PAA measurements before and after dialyzer reprocessing should be at 2,000 ppm and 800 ppm, respectively.

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