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Green Betel Leaf and Lemongrass Extracts as Sustainable Disinfectants – Optimizing Dosage and Environmental Impact for Medical Waste Recycling

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ABSTRACT

This study investigates the efficacy of green betel leaf (*Piper betle Linn*) and lemongrass (*Cymbopogon citratus*) extracts as natural disinfectants in the medical waste recycling process in Indonesia. The research aims to provide a sustainable alternative to chemical disinfectants, such as chlorine, by optimizing the dosage and contact time for effective microbial reduction. The study focused on the disinfection of *Bacillus subtilis* and *Staphylococcus* as bioindicators, evaluating the impact of varying disinfectant concentrations on key environmental parameters, including pH, temperature, and total dissolved solids (TDS). The findings indicate that a 0.5% concentration with a 30-minute contact time is optimal for reducing *Bacillus subtilis*, while higher concentrations are required for effective *Staphylococcus* reduction. Additionally, the study reveals that higher disinfectant concentrations lower pH and increase TDS, posing potential challenges for wastewater management. These results highlight the potential of natural disinfectants to enhance the sustainability of medical waste management practices in Indonesia, though further research is needed to address the environmental and regulatory challenges associated with their use.

Keywords: natural disinfectants; medical waste management; recycling; green betel leaf; lemongrass; sustainability.

INTRODUCTION

Medical waste management is a critical aspect of maintaining public health and environmental sustainability, particularly within healthcare facilities where hazardous waste poses significant risks (Anwar et al., 2013). Effective management of this waste is essential to prevent the spread of infectious diseases and minimize environmental contamination, which is increasingly important as the volume of medical waste grows due to events like the COVID-19 pandemic (Kalantary et al., 2021; Nindrea et al., 2021). In Indonesia, the implementation of stringent regulations and the adoption of sustainable waste management practices are necessary to address these challenges, highlighting the need for ongoing improvements in this field (Ibrahim, 2022).

The disinfection of medical waste is a crucial step in waste management, traditionally dominated by chemical disinfectants like chlorine. However, the use of chlorine has raised concerns due to the potential formation of toxic disinfection byproducts (DBPs) that pose risks to both human health and the environment (Wang et al., 2020). These concerns have led to an exploration of alternative disinfectants that can offer effective pathogen elimination without the associated risks, thereby enhancing the safety and sustainability of medical waste management practices (Dang et al., 2023).

The reliance on chlorine as a disinfectant in medical waste management, while effective in pathogen control, has significant drawbacks, particularly the formation of harmful DBPs that can contaminate the environment and pose long-term health risks (Huitric et al., 2014). The increasing recognition of these issues has spurred research into alternative disinfectants that can mitigate these risks while maintaining efficacy. This shift towards more sustainable practices is crucial, given the growing global emphasis on environmental protection and public health.

One promising solution is the use of natural disinfectants derived from plant extracts, such as green betel leaf (*Piper betle Linn*) and lemongrass (*Cymbopogon citratus*), which have demonstrated strong antimicrobial properties (Fikri et al., 2021). These natural extracts offer a potential alternative to chemical disinfectants, providing effective pathogen control without the environmental and health hazards associated with synthetic chemicals. Their application in the medical waste recycling process could significantly reduce the risks associated with chlorine use, aligning with global efforts to improve waste management practices in healthcare facilities.

Research has shown that green betel leaf and lemongrass extracts contain phenolic compounds, such as catechol, pyrogallol, quinone, and eugenol, which are known for their potent antimicrobial activities (Fikri et al., 2021). These compounds inhibit microbial enzyme activity, making them effective against a wide range of pathogens, including *Bacillus subtilis* and *Bacillus stearothermophilus*, which are commonly used as bioindicators in disinfection studies (Fikri et al., 2021). The application of these extracts in disinfection processes offers a natural, biodegradable alternative to chlorine, reducing the formation of harmful byproducts and minimizing environmental impact (Anggraini and Lestari, 2022). Furthermore, the antimicrobial efficacy of these natural extracts has been validated in various studies, which demonstrate their ability to achieve pathogen reduction comparable to that of chemical disinfectants like chlorine (Anicetus et al., 2022). The use of these natural disinfectants also addresses the increasing demand for sustainable waste management practices, which are essential for mitigating the environmental impacts of healthcare facilities (Huang et al., 2020). By replacing chlorine with plant-based extracts, healthcare facilities can enhance their waste management protocols while adhering to environmental regulations and public health standards (Dang et al., 2023).

Comparatively, the adoption of green betel leaf and lemongrass extracts as disinfectants aligns with the principles of green chemistry, which emphasizes the use of environmentally friendly substances in industrial and healthcare processes (Elsaidy et al., 2022). This approach not only ensures effective disinfection but also supports the broader goal of reducing chemical pollution and promoting ecological health. As such, these natural extracts represent a significant advancement in the field of medical waste management, offering a viable and sustainable alternative to traditional chemical disinfectants (Dang et al., 2023).

Despite the promising results from studies on the use of natural extracts as disinfectants, there remains a gap in the literature regarding the optimization of their use, particularly in terms of dosage and contact time (Fikri et al., 2021). While the antimicrobial properties of green betel leaf and lemongrass extracts have been established, there is limited research on their application in large-scale medical waste recycling processes. Specifically, the ideal concentrations and exposure durations required to achieve consistent and effective disinfection need further investigation to ensure these natural disinfectants can replace chlorine without compromising safety or efficacy (Anicetus et al., 2022). Moreover, while these studies have demonstrated the potential of natural disinfectants to reduce environmental impact, there is a need for comprehensive evaluations of their long-term effects on human health and the ecosystem (Anggraini and Lestari, 2022). Current research has primarily focused on the immediate antimicrobial efficacy of these extracts, leaving a gap in understanding their broader environmental and health implications when used extensively in medical waste management (Elsaidy et al., 2022). Addressing these gaps is critical for the development of guidelines and standards for the use of natural disinfectants in healthcare facilities.

Lastly, there is a paucity of comparative studies that directly evaluate the effectiveness of these natural disinfectants against the traditional chlorine-based methods across different types of medical waste and pathogens (Huang et al., 2020). Such studies are essential to establish the reliability and generalizability of these natural extracts in diverse healthcare settings. Without this comparative analysis, the full potential of green betel leaf and lemongrass extracts as safe and effective alternatives to chlorine in medical waste recycling cannot be fully realized (Dang et al., 2023). This study aims to investigate the efficacy of green betel leaf (*Piper betle Linn*) and lemongrass (*Cymbopogon citratus*) extracts as natural disinfectants in the medical waste recycling process, with a particular focus on optimizing dosage and contact time. The novelty of this research lies in its exploration of these natural extracts as viable alternatives to chlorine, a common but potentially hazardous chemical disinfectant. By determining the optimal conditions for their use, this study seeks to provide a safer, more sustainable solution for disinfecting medical waste, contributing to the broader field of environmental health and waste management.

The scope of this research includes a comparative analysis of the antimicrobial efficacy of these natural extracts against *Bacillus subtilis* and *Bacillus stearothermophilus*, two key bioindicators used in disinfection studies. Additionally, the study will evaluate the environmental impact of using these natural disinfectants, considering their potential to reduce harmful disinfection byproducts. The findings will be applicable to healthcare facilities seeking to adopt greener waste management practices while maintaining high standards of public health and environmental protection.

METHODS

The materials used in this study include green betel leaf (*Piper betle Linn*) and lemongrass (*Cymbopogon citratus*), both selected for their known antimicrobial properties, specifically targeting *Bacillus subtilis* and *Bacillus stearothermophilus*. The plant materials were sourced from local farms to ensure freshness and potency (Fikri et al., 2021). Additionally, distilled water, ethanol, and standard microbiological media were used for the extraction process and cultivation of bacterial cultures, respectively. The choice of these materials is grounded in previous research indicating their efficacy as natural disinfectants.

The samples for this study were prepared through a meticulous process. The green betel leaf and lemongrass were thoroughly washed with distilled water to remove any impurities, then air-dried at room temperature. Once dried, the leaves were finely ground into powder form using a mechanical grinder. This powder was subjected to ethanol extraction, wherein 100 grams of plant material was soaked in 500 ml of 70% ethanol solution for 48 hours, followed by filtration (Fikri et al., 2021). The filtrate was then evaporated to obtain concentrated extracts, which were stored in sterilized glass containers under refrigeration until further use.

The experimental setup involved the use of a factorial randomized design to test the efficacy of the green betel leaf and lemongrass extracts as disinfectants. The setup included a series of reaction vessels where different dosages of the extracts were tested against *Bacillus subtilis* and *Bacillus stearothermophilus*. Each reaction vessel contained a fixed amount of medical waste, previously sterilized and inoculated with known concentrations of the bacterial cultures. The disinfectant solution was then added, and the samples were incubated for specified contact times (15, 30, and 45 minutes) to determine the optimal conditions for bacterial reduction (Anggraini and Lestari, 2022). The reaction vessels were equipped with temperature controls to maintain consistent experimental conditions, ensuring the reliability of the results.

The primary parameters measured in this study were the reduction in bacterial load of *Bacillus subtilis* and *Bacillus stearothermophilus* following treatment with the plant extracts. Bacterial counts were determined using the plate count method, which involves serial dilution and culturing of the treated samples on nutrient agar plates. The bacterial colonies were counted after incubation at 37 °C for 24 hours. The effectiveness of the extracts was compared to that of a standard chlorine-based disinfectant used as a control. Additionally, the study measured the chemical composition of the extracts using gas chromatography-mass spectrometry (GC-MS) to identify the active compounds responsible for the antimicrobial activity.

Statistical analysis was conducted using a two-way ANOVA to assess the impact of dosage and contact time on the bacterial reduction efficacy of the disinfectants. The analysis aimed to determine any significant interactions between these factors and to identify the optimal conditions for disinfection. Post-hoc tests were performed to compare the mean differences among the treatment groups, and results with a p-value of less than 0.05 were considered statistically significant. The statistical software SPSS version 18.0 was utilized for data analysis, ensuring a rigorous evaluation of the experimental outcomes.

RESULTS AND DISCUSSION

Measurement results of Bacillus subtilis in the medical waste recycling process

Bacillus subtilis is a Gram-positive, rod-shaped bacterium that is widely found in soil and the gastrointestinal tract of ruminants and humans (Fig. 1) (Logan and Rodríguez-Díaz, 2006). This characteristic makes *Bacillus subtilis* particularly important in various industrial and environmental processes, including medical waste recycling. In the medical waste recycling process, *Bacillus subtilis* plays a crucial role due to its ability to survive harsh conditions through spore formation, its enzymatic capabilities in breaking down organic matter, and its general safety as a non-pathogenic bacterium (Thakur et al., 2014). Its resilience and biodegradative properties make it a valuable organism in ensuring that medical waste is effectively processed and recycled, contributing to the overall safety and sustainability of waste management systems.

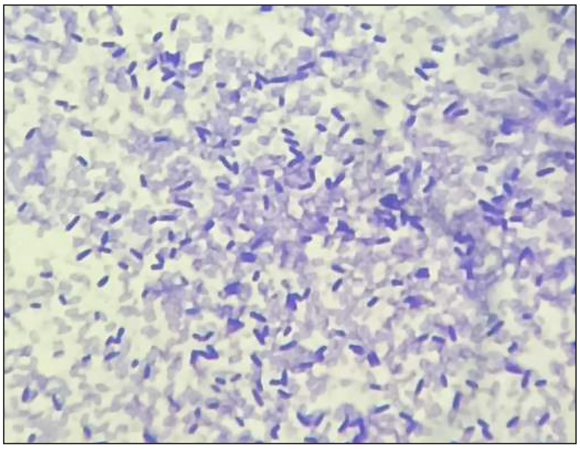

Figure 1. *Bacillus subtilis* (1000×)

The study analyzed the impact of varying disinfectant concentrations and contact times on the bacterial load of *Bacillus subtilis* during the medical waste recycling process. Table 1 illustrates that the disinfectant's effectiveness increased with both concentration and contact time, particularly at a concentration of 0.5%, which was the most effective across all tested intervals. At 30 minutes, the bacterial count was significantly reduced, nearly eradicating *Bacillus subtilis*. Higher concentrations (e.g., 3%, 5%, and 7%) also showed a reduction in bacterial load but did not consistently outperform the 0.5% concentration, especially in shorter contact times. The findings suggest that a disinfectant concentration of 0.5% with a contact time of 30 minutes is optimal for reducing *Bacillus subtilis* effectively.

The findings align with previous studies on the efficacy of natural disinfectants, such as those by Fikri et al. (2021), who also observed significant bacterial reduction using plant extracts. However, the current study expands on this by identifying the optimal concentration and contact time for *Bacillus subtilis* specifically, contributing to the ongoing discourse on sustainable disinfection practices (Elsaidy et al., 2022). The effectiveness of 0.5% disinfectant concentration is particularly notable as it offers a balanced approach between efficacy and environmental impact, contrasting

Table 1. The number of *Bacillus subtilis* based on disinfection variations and contact time duration

Concentration	Number of *Bacillus subtillis*		
	Contact time (15 minute)	Contact time (30 minute)	Contact time (45 minute)
0.03	931	2568	799
0.05	691	387	1050
0.07	424	35	601
0.3	130	502	413
0.5	9105	17	554
0.7	2057	52	870
3	17613	1027	129
5	7271	309	1666
7	2876	16452	149
Min	130	17	129
Max	17613	16452	1666
Average	4567	2372	692
St. Dev	5834.90	5340.49	479.29

with higher concentrations that may contribute to increased TDS levels and potential environmental harm (Huang et al., 2020). Additionally, while *Bacillus subtilis* was effectively reduced, the resilience of *Staphylococcus* in this study suggests that different bacteria respond variably to the same treatment, highlighting the need for tailored disinfection strategies (Anggraini and Lestari, 2022).

The findings underscore the potential of natural disinfectants in reducing *Bacillus subtilis* in medical waste, presenting a viable alternative to traditional chemical disinfectants like chlorine. The optimal use of a 0.5% concentration with a 30-minute contact time not only ensures effective disinfection but also aligns with global efforts to minimize the environmental impact of waste management (Dang et al., 2023). This study's results could inform healthcare facilities in adopting greener practices without compromising public health standards. Moreover, the insights gained from this research could drive the development of guidelines for the broader application of natural disinfectants in medical waste management, ultimately contributing to safer and more sustainable environmental practices (Anicetus et al., 2022; Fikri et al., 2021).

Findings of *Staphylococcus* in the medical waste recycling process

Staphylococcus is a genus of Gram-positive bacteria, known for their round (cocci) shape and tendency to form clusters resembling grape-like bunches (Fig. 2) (Pal et al., 2021). These bacteria are ubiquitous in the environment and are commonly found on the skin and mucous membranes of humans and animals (Faccin et al., 2023). The genus includes several species, with *Staphylococcus aureus* being one of the most well-known due to its role as both a commensal organism and a pathogen.

In the context of medical waste recycling, *Staphylococcus* poses significant challenges due to its ability to resist disinfectants and its potential to cause infections. Its capability to form biofilms and survive harsh conditions makes it essential to ensure that waste management processes are thorough and effective in eliminating these bacteria. Proper sterilization and handling of medical waste contaminated with *Staphylococcus* are critical to preventing the spread of these potentially dangerous organisms (Padmanabhan and Barik, 2019; Rutala and Weber, 2015). The analysis indicates that the effectiveness of the

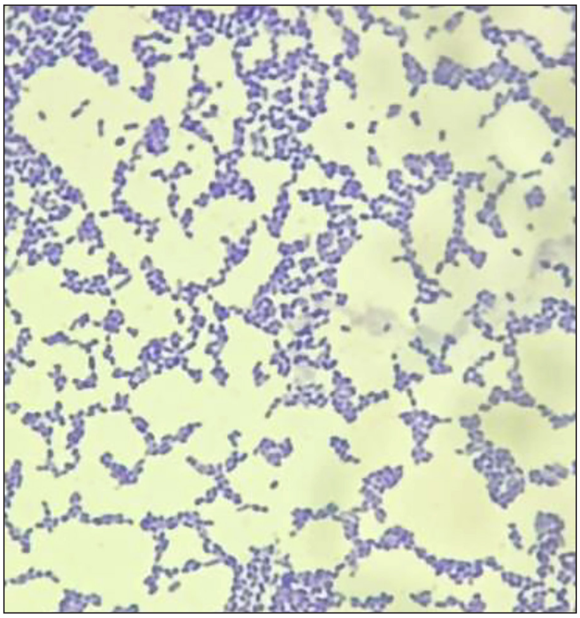

Figure 2. *Staphylococcus* (1000×)

disinfectant in reducing *Staphylococcus* bacteria varies depending on the concentration and contact time. For a shorter contact time of 15 minutes, a concentration of 0.7% is most effective. At 30 minutes, a low concentration of 0.05% shows the best reduction, demonstrating that even low concentrations can be highly effective with sufficient exposure time (Table 2).

However, at a 45-minute contact time, a high concentration of 7% yields the most significant reduction, indicating that prolonged exposure to higher concentrations is beneficial. Interestingly, certain concentrations like 0.5% show varying effectiveness across different time points, which may suggest complex interactions between the bacteria and the disinfectant at those levels. In summary, to achieve the most significant reduction in *Staphylococcus*, a 7% concentration with a 45-minute contact time is recommended. For shorter contact times, a 0.7% concentration (15 minutes) or 0.05% concentration (30 minutes) are also effective options (Table 2).

In the study on the recycling process of medical waste using green betel leaf extract and lemongrass as disinfectants, the initial aim was to evaluate the effectiveness of these natural agents against specific bioindicators, namely *Bacillus subtilis* and *Bacillus stearothermophilus*. These bacteria were chosen for their known resilience and capacity to survive harsh conditions, making them ideal candidates for assessing the efficacy of disinfection processes (Li et al., 2023; Martin, 2009). However, the

Table 2. The number of *Staphylococcus* based on disinfection variations and contact time duration

Concentration	Number of *Staphylococcus*		
	Contact time (15 minute)	Contact time (30 minute)	Contact time (45 minute)
0.03	645	1978	305
0.05	5185	111	468
0.07	561	104	6427
0.3	779	159	727
0.5	8734	509	15227
0.7	229	252	1335
3	148	6058	14855
5	3546	9023	1953
7	5272	3484	102
Min	148	104	102
Max	8734	9023	15227
Average	2789	2409	4600
St. Dev	3060.95	3207.88	6223.55

results of the study revealed the presence of *Bacillus subtilis* but not *Bacillus stearothermophilus*. Unexpectedly, another bacterial species, *Staphylococcus*, was detected instead.

The absence of *Bacillus stearothermophilus* in the study could be attributed to several factors. First, it is possible that the disinfection process using green betel leaf extract and lemongrass was particularly effective against *Bacillus stearothermophilus*. This bacterium, while known for its heat resistance, may not have been able to survive the specific antimicrobial properties of the betel leaf and lemongrass extracts, particularly their phenolic compounds and essential oils, which have been shown to exhibit strong antibacterial activities (Madhumita et al., 2019; Pranoto et al., 2021). The conditions set during the disinfection process (such as concentration, exposure time, and environmental conditions) may have been optimal for eradicating *Bacillus stearothermophilus*, hence its absence in the final analysis.

Conversely, the persistence of *Bacillus subtilis* suggests that this bacterium may have certain resilience against the disinfection methods employed. *Bacillus subtilis* is known for its ability to form endospores, which can withstand harsh environments, including exposure to various antimicrobial agents (Checinska et al., 2015; Zhang et al., 2020). The conditions in the study might have been sufficient to inactivate or destroy some microbial species, yet *Bacillus subtilis* survived due to its spore-forming ability. It is possible that the specific mode of action of the natural extracts used did not completely penetrate or disrupt the protective endospore layer of *Bacillus subtilis*, allowing it to persist post-treatment.

The detection of *Staphylococcus* was unexpected, as it was not one of the targeted bioindicators. The presence of *Staphylococcus* could be due to contamination during the handling or processing of the medical waste or during sample collection and analysis (Hossain et al., 2013; Susan et al., 2018). *Staphylococcus* species, particularly *Staphylococcus aureus*, are common in the environment and on human skin, making them potential contaminants (Kozajda et al., 2019). Additionally, if the disinfection process was more effective against Gram-positive spore-forming bacteria (like *Bacillus stearothermophilus*), it might have inadvertently allowed the non-spore-forming *Staphylococcus* to proliferate in the absence of competition, especially if the disinfectant was less effective against *Staphylococcus* due to biofilm formation or inherent resistance mechanisms.

The results of the study highlight the complexity of microbial dynamics in the medical waste recycling process. The absence of *Bacillus stearothermophilus* and the presence of *Staphylococcus* suggest that while the disinfection method using green betel leaf extract and lemongrass was effective against certain bioindicators, it may not be universally effective across all microbial species. The persistence of *Bacillus subtilis* and the emergence of *Staphylococcus* point to the need for a more comprehensive analysis of the disinfectant's spectrum of activity, as well as strict contamination control measures during the study. Future research may focus on optimizing the disinfection process to ensure the complete eradication of all target microorganisms while preventing the introduction or proliferation of unintended species like *Staphylococcus*.

The influence of pH, temperature, and TDS on the disinfection process of medical waste recycling

The pH decreases consistently with increasing disinfectant concentration across all contact times. The most noticeable drop in pH occurs at concentrations of 0.5% and higher. Specifically, a concentration of 0.7% at 45 minutes results in a pH of 5.6, which is among the lowest values observed (Fig. 3). This suggests that for reducing pH (acidifying the solution), higher concentrations and longer contact times are most effective. This drop in pH, especially to values as low as 5.6 at 45 minutes, suggests that natural disinfectants can alter the chemical environment of the waste, potentially enhancing the antimicrobial effectiveness of the solution by disrupting microbial cell membranes which are more susceptible in acidic conditions (Elsaidy et al., 2022).

Interestingly, Figure 4 reveals that the temperature remained relatively stable across all concentrations and contact times, with only slight

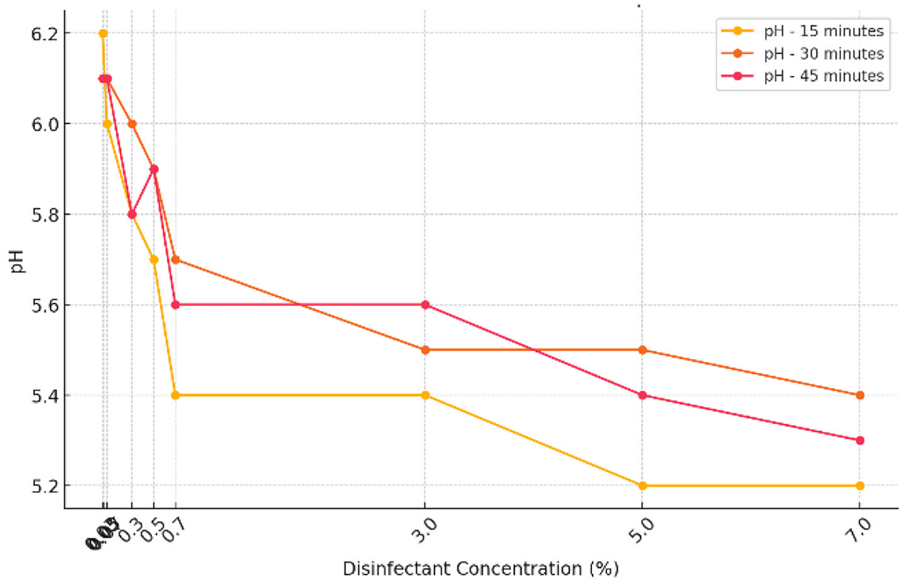

Figure 3. Effect of disinfectan concentration on pH over time

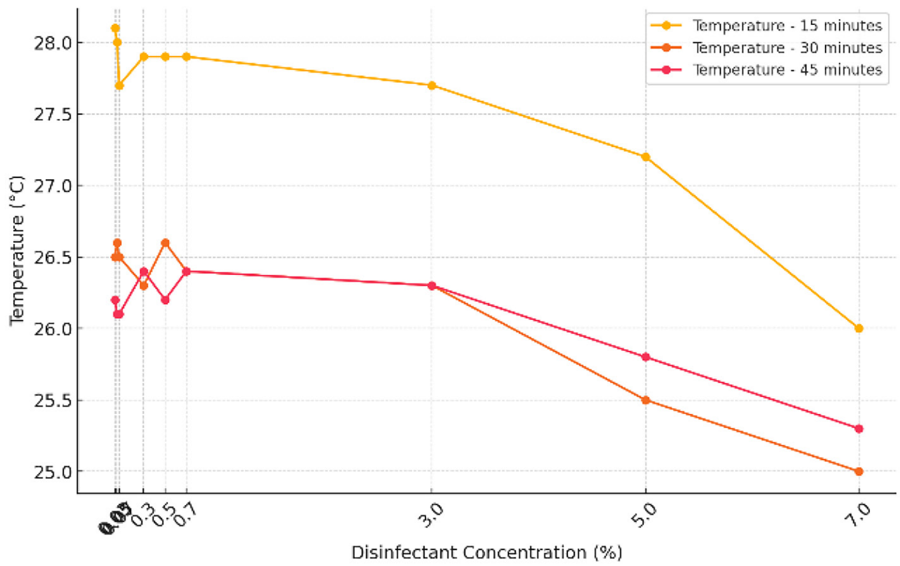

Figure 4. Effect of disinfectan concentration on temperature over time

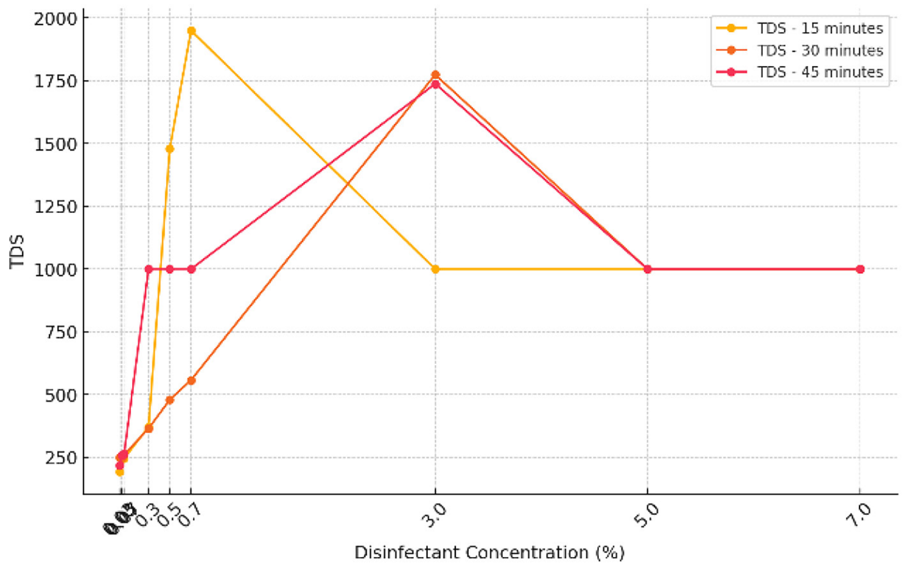

Figure 5. Effect of disinfectan concentration on TDS over time

decreases observed at higher concentrations, such as 7% at 45 minutes. The minimal variation in temperature suggests that while disinfectant concentration impacts pH significantly, it does not markedly influence the thermal conditions of the process. This stability is advantageous as it prevents thermal degradation of the disinfectants, thereby preserving their efficacy over extended contact times (Huang et al., 2020)

The impact on TDS, as illustrated in Figure 5, is particularly noteworthy. TDS levels increased sharply with both higher disinfectant concentrations and longer contact times, often exceeding 1000 at concentrations of 0.5% and above after 45 minutes. This significant increase indicates a substantial accumulation of dissolved solids in the solution, likely due to the breakdown of organic and inorganic matter within the waste as the disinfectants act on the microbial content (Michael-Kordatou et al., 2015). Elevated TDS levels can be indicative of the disinfectants' effectiveness in solubilizing waste components, but they also suggest potential challenges for wastewater management post-disinfection, as high TDS can complicate water treatment processes (Fikri et al., 2021)

The observed trends in pH, temperature, and TDS underline the complexity of optimizing disinfectant concentrations for medical waste treatment. A concentration of 0.7% emerges as a critical threshold, beyond which the solution becomes significantly more acidic and dense with dissolved solids, potentially enhancing microbial reduction but also posing challenges for waste management. The relatively stable temperature across concentrations is reassuring, as it allows for flexible disinfection protocols without the need for additional thermal regulation.

These findings suggest that natural disinfectants, when applied at optimal concentrations, can effectively manage pH and TDS levels to enhance the disinfection process while maintaining environmental stability. However, the increase in TDS with higher concentrations calls for careful monitoring to avoid potential downstream impacts on wastewater systems. Overall, this study contributes valuable data for the development of balanced, sustainable disinfection strategies in medical waste management that optimize microbial control while mitigating environmental risks (Anggraini and Lestari, 2022).

CONCLUSIONS

This study demonstrates the potential of natural disinfectants, specifically green betel leaf and Lemongrass extracts, as effective alternatives to traditional chemical disinfectants in the medical waste recycling process in Indonesia. The findings indicate that these natural agents can significantly reduce microbial loads, such as *Bacillus subtilis*, while also impacting key environmental parameters like pH and TDS. The use of these natural disinfectants offers several opportunities, including the promotion of sustainable waste management practices, enhancement of public health, and economic benefits from local sourcing.

However, challenges remain in optimizing disinfection processes, managing environmental impacts, and addressing regulatory and scalability issues. Future research is essential to refine the application of these natural disinfectants, ensuring their effectiveness across a broader range of pathogens and settings. By addressing these challenges, natural disinfection could become a pivotal strategy in advancing Indonesia's medical waste management practices towards greater sustainability and public health protection

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