DOI: 10.24996/ijs.2024.65.11.9

ISSN: 0067-2904

Potency of Face Mask-Degrading Bacteria Isolated from Parangtritis Beach, Yogyakarta, Indonesia

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Received: 7/12/2022 Accepted: 5/9/2023 Published: 30/11/2024

Abstract

During the COVID-19 epidemic, improperly discarded face masks turned out to be a severe source of environmental contamination that threatened lives. Bacteria are a crucial degrading agent for face masks. The objective of this study was to isolate and characterize potential face mask-degrading bacteria from discarded face masks at Parangtritis Beach in Yogyakarta, Java, Indonesia. Mineral Salt Media (MSM) containing face mask (black duckbill, blue surgical and white KF94) was used to select bacterial growth ability. The process of face mask degradation was also assisted by scanning electron microscopy (SEM) examination. Eight bacterial strains, designated AP1 through AP8, were obtained using an enrichment screening technique. AP1-AP3, AP4-AP5, and AP6-AP8 were identified phenotypically as *Bacillus* sp., *Pseudomonas* sp. and *Staphylococcus* sp. respectively. *Bacillus* sp. (AP1) displayed the highest growth (OD_{600} 0.78) on all test conditions among the eight isolates. Moreover, this strain could be grown on MSM-face mask media with a pH range of 5 - 9; temperature 5 – 50°C, and NaCl concentration of 2.5 - 7.5%. The SEM study revealed morphological alterations in the face masks inner, middle and outer layers after interaction with *Bacillus* sp. This research proved that *Bacillus* strains could be viable candidates for face mask biodegradation.

Keywords: *Bacillus*, Degradation, Disposal mask, Isolation, Selection.

Introduction

The global COVID-19 pandemic increased the need for face masks for single usage [1] which led to the increase in face masks wastes produced from hospitals and housholds, damaging the environment or health [2]. Since the beginning of the epidemic, discarded face masks were observed in nearly every terrestrial and aquatic ecosystems [3]. However, neither the initial creation of face mask wastes nor the implications of solid waste management for preventing environmental pollution were understood [4]. An estimated 0.15 million to 0.39 million tons of plastic wastes from face masks was expected to wind up in the waters of the world within the first year [5]. Face masks can get entangled in the marine life, poison and suffocate corals and propagate invasive species [6]. The improper disposal of worn face masks created a new form of non-biodegradable plastic waste that could take hundreds of years to decompose [7].

Face masks contain microplastics, heavy metals and organic contaminants [8]. In addition, most single-use masks comprise of thermoplastic polymers such as polyethylene terephthalate; polyethylene (PE) with high, low and linearly low densities; chloride polymer;

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polypropylene (PP); polystyrene; nylons; polyamide; and polylactic acid. PP and PE are the two most commonly found polymers in face masks [9]. Microplastics and microfiber, which contaminate water and soil, are formed when discarded face masks undergo physical and chemical breakdown in the environment [10]. The likelihood that ambient microplastic concentrations would increase, hence increasing the likelihood of food web interaction, ingestion, and adverse impacts [11] raised an urgent need for resourceful and environmentally friendly waste management.

Dire need for developing new strategies that could provide ecologically acceptable and highly selective face mask deterioration measures was felt. There was a growing trend of utilizing indigenous microbes in face mask biodegradation due to their eco-friendliness, greater specificity, suitability for in situ procedures, and potential for genetic engineering development [12]. However, a systematic and reproducible examination of the biodegradation process required the isolation of bacteria as pure cultures. In addition, isolation is suitable for the identification and in-depth research of bacteria that have been supplemented with discarded face mask.

Parangtritis Beach, a coastal tourism area in the southern part of Java, Yogyakarta, Indonesia, showed extensive face mask pollution which allowed the potential of indigenous bacteria to adapt to a face mask environment. However, face mask-degrading bacteria are uncommon in environmental samples; therefore, their population must be augmented prior to separation. This study aimed to isolate, identify and select indigenous bacteria capable of degrading disposed face masks from Parangtritis Beach. Phenotypic characterization was performed to identify the bacterial isolates, and the capacity to grow in MSM-facial mask media which served as a marker for face mask-degrading bacteria. In addition, SEM analysis was conducted to examine the face mask morphological changes. The face mask wastes were gathered from the coasts around the beach to investigate correlation between environmental conditions and the dispersion of bacteria on face masks. Consequently, this analysis showed the soil elemental composition and physicochemical properties. This study intended to highlight the significance of indigenous bacteria to encourage more research on face mask degradation.

Materials and Methods

Bacterial Isolation and Characterization

This research was conducted at Universitas Negeri Yogyakarta's Microbiology Laboratory, Faculty of Mathematics and Natural Sciences. In May 2022, three samples of disposable face masks (black duckbill, white duckbill, and blue surgical) were collected from Parangtritis Beach in Yogyakarta, Java, Indonesia (8°01'28.4"S 110°19.47"E) for the isolation of bacteria. Face mask-degrading bacterial communities were conducted by enrichment screening using the Mineral Salt Medium (MSM). Ten grams of three sample were dissolved in 90 mL of MSM with the following composition (g/L), i.e.: KH_2PO_4 0.5, K_2HPO_4 1.5, NH_4NO_3 1.0, NaCl 1.0, and $MgSO_4 \cdot 7H_2O$ 0.1 and then adjusted to pH 7.0 [13]. Time incubation was conducted for one week at room temperature in a reciprocating shaking at 100 rpm. One mL of enrichment culture was diluted adequately with sterile saline (0.8%) and spread on the Luria Bertani/LB (tryptone 10 g/L, yeast extract 5 g/L, and NaCl 10 g/L) agar plates. Incubation was carried out at 30°C for 72 h. Pure cultures of the bacterial isolates were obtained by repeated sub-culturing of individual colonies formed on LB agar. The identification of bacterial strain was performed according to Bergey's Manual of Determinative Bacteriology [14].

Bacterial Selection

The bacterial face mask degrading ability can be determined with the growth measurements. Moreover, growth measurements were carried out based on the value of optical density (OD) with a spectrophotometer at 600 nm wavelength. Bacterial pure cultures were tested for their ability to grow on MSM-containing face masks. The face masks were obtained from the local market. Inoculum preparation was conducted by inoculating bacteria in LB medium for 24 hours at 30°C. Moreover, bacterial inoculum size of OD_{600} 0.8 was used. Aseptically, 10% bacterial inoculums were inoculated into 5 mL MSM-containing 5 mm diameter of the face masks (black duckbill, blue surgical, white KF94) which were then incubated at 30°C for 24 hours without shaking. Static incubation conditions were maintained to reduce mask degradation due to agitation.

Growth Profile of Selected Bacterial Strains

The potential of bacteria for degradation can also be determined by environmental factors. Therefore, measurements of the bacterial growth curve were carried out at various conditions of pH, temperature and NaCl levels. The culture growth was determined by measuring sample turbidity using a spectrophotometer (GENESYS 10S, Thermo Fisher Scientific) at 600 nm every 3 h for 24 h on different face mask type (black duckbill, blue surgical, white KF94) at different temperatures (5, 30, and 50 °C) under pH 5, 7, and 9., and NaCl concentration of 2.5; 5.0; 7.5%. All experiments were carried out using MSM media and bacterial inoculum size of OD_{600} 0.8 in a static condition. Inoculum preparation was conducted by inoculating bacteria in LB medium for 24 hours at 30°C.

Scanning Electron Microscopy (SEM) Analysis

This investigation was conducted to evaluate the face mask's exterior, middle and inner morphology after 24 hours of contact with bacteria in MSM media without shaking. The samples were then put onto pellets in a copper grid covered with carbon for examination by SEM (JSM-6510LA, Japan).

Soil Physicochemical Properties

The soil surrounding the disposed face mask was collected to characterize the environmental condition as the disposal face masks on the beach were buried or embedded in it. Soil physicochemical parameters determined the presence of bacteria in the disposal face mask on the beach. This analysis was carried out at *Balai Besar Teknik Kesehatan Lingkungan dan Pengendalian Penyakit* (BBTKLPP) Yogyakarta and *Balai Pengkajian Teknologi Pertanian* (BPTP) Yogyakarta. A standard approach was used to test soil physiochemical parameters, including texture, pH, salinity, moisture level, contents of organic matter (OM), organic carbon (OC), total nitrogen (TN), phosphorus (P), potassium (K), C/N ratio and cation exchange capacity (CEC).

Results and Discussion

Isolation and Identification of Bacteria

All disposable face masks found around Parangtritis Beach were wastes which demonstrated that their improper disposal endangers human life. Marine plastic pollution was exacerbated by the prevalence of thrown away face masks [15]. On Parangtritis Beach, numerous types of disposable face masks, included black duckbill, white duckbill, and blue surgical masks, were collected (Figure 1). The findings of bacterial isolation from three types of disposable face masks yielded eight isolates, designated AP1 through AP8, that differed based on color, edge, colony shape, and elevation. These isolates were then purified using the same media and incubated for 48 hours. Identification of bacteria was conducted according to their morphological, cultural, physiological, and biochemical properties. *Bacillus* sp., *Pseudomonas* sp., and *Staphylococcus* sp. were identified phenotypically as being AP1-AP3, AP4-AP5, and AP6-AP8, respectively. *Bacillus* and *Staphylococcus* were found in all three samples, while *Pseudomonas* was found in blue surgical and black duckbill. *Bacillus* was rod-shape, gram-positive bacteria that could be isolated from a river [16]; mangrove [17]; coastal region [18]; radiation and heavy metal-polluted soil [19]; plastic dump site [20]; and dump soil [21]. A previous study isolated rod-shape and gram-negative *Pseudomonas* bacteria from freshwater [22]; soil [23]; hot water spring [24]; mangrove [25]; and marine [26]. Other previous investigations of gram-positive cocci *Staphylococcus* revealed their presence in skin [27]; cell phone [28]; marine [29]; and soil [30].

Figure 1: Examples of disposal face mask in Parangtritis beach.

Bacterial Selection

The face mask biodegradability tests were conducted on media without a carbon source but providing essential mineral ingredients for the bacteria. In this work, we used three types of face masks for bacterial selection: the black duckbill mask, the blue surgical mask and the white KF94 mask. Regarding the bacteria ability to break down face masks when they have developed in a medium containing face masks as the sole carbon source, it is assumed that they can do so. A previous study has demonstrated that the polymer is degraded when bacteria develop in a polyethylene-containing medium [31]. The maximal growth rate and cell density of bacteria in media containing different face mask types is demonstrated in Figure 2. Different values of OD indicate that the biomass growth of these bacterial isolates was highly affected by the presence of the face mask in the growth medium. After 24 h of incubation, the order of cell growth for all eight isolates was found to be *Bacillus* sp. (AP1) >*Bacillus* sp. (AP2) >*Bacillus* sp. (AP3) >*Pseudomonas* sp. (AP4) >*Pseudomonas* sp. (AP5) >*Staphylococcus* sp. (AP5) > *Staphylococcus* sp. (AP6) at MSM-face mask media which suggested that *Bacillus* sp. (AP1) could potentially degrade the mask and could be used as a substrate for growth. In line with other studies, it was also demonstrated that *Bacillus* is a plastic degrader [32][33][34]; polyethylene degrader [35]; and azo dye decolorizer [36].

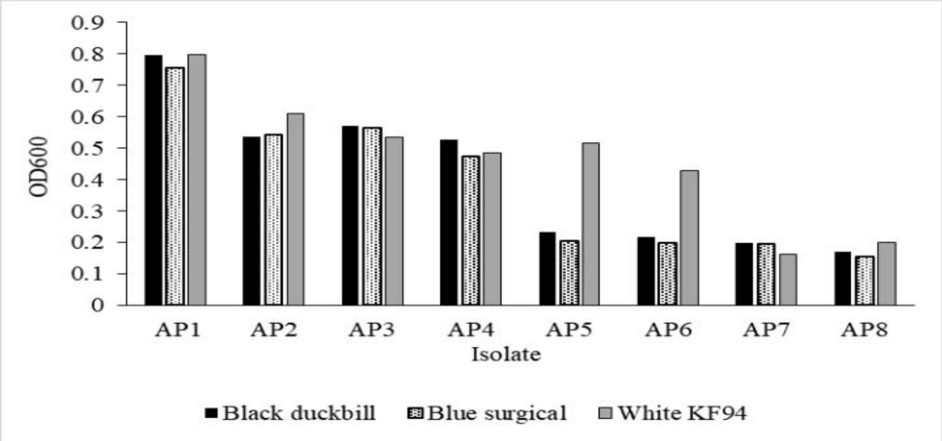

Figure 2: Bacterial growth at MSM-face mask media.

Bacterial Growth Profile

Bacillus sp. (AP1) was selected to study the effects on environmental condition, i.e.: pH, temperature, and NaCl concentration. The growth curve of all conditions was characterized by log phase of up to 3 h. It was observed that this strain was able to grow at pH of 5, 7 and 9 (Figure 3). This strain when grew at three different temperatures (5, 30, and 50°C) for 24 h showed similar growth responses (Figure 4). Moreover, changes in NaCl concentration of 2.5, 5.0, 7.5% did not affect the growth of this strain (Figure 5). From the growth curve pattern, it is suggested that these bacteria exhibited a strong tolerance towards pH, temperature and salinity stress.

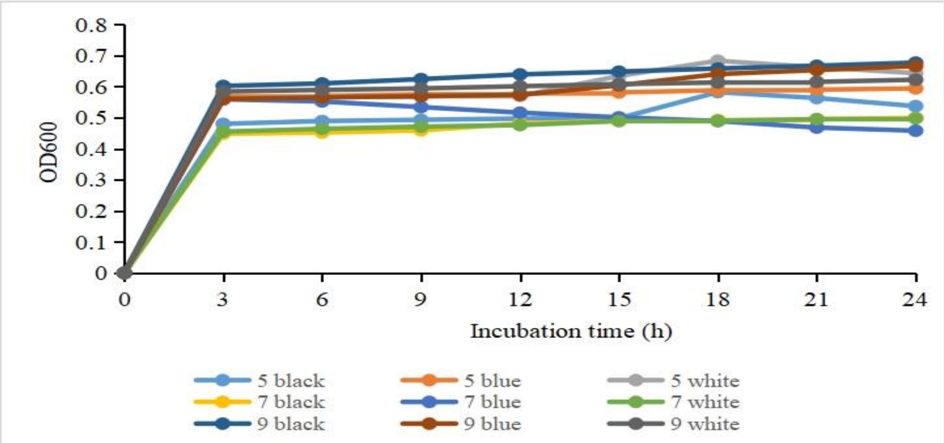

Figure 3: *Bacillus* sp. AP1 growth at MSM-face mask media under different pH (5, 7 & 9).

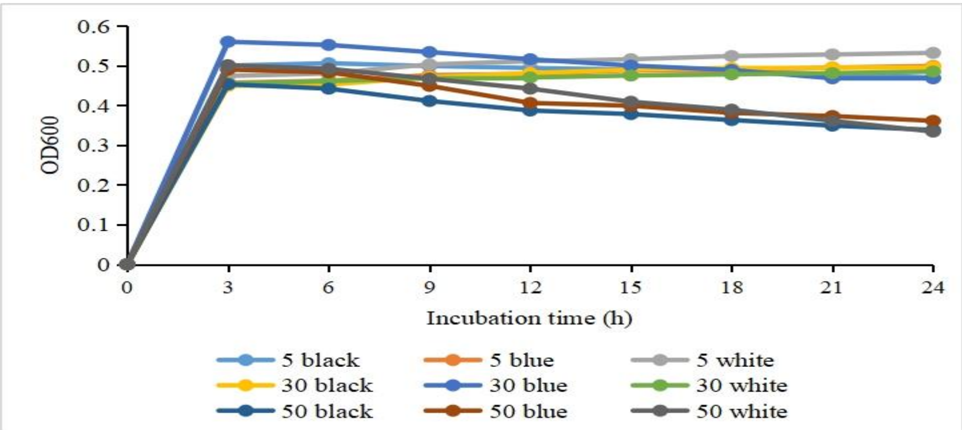

Figure 4: *Bacillus* sp. AP1 growth at MSM-face mask media under different temperatures (5, 30, 50°C).

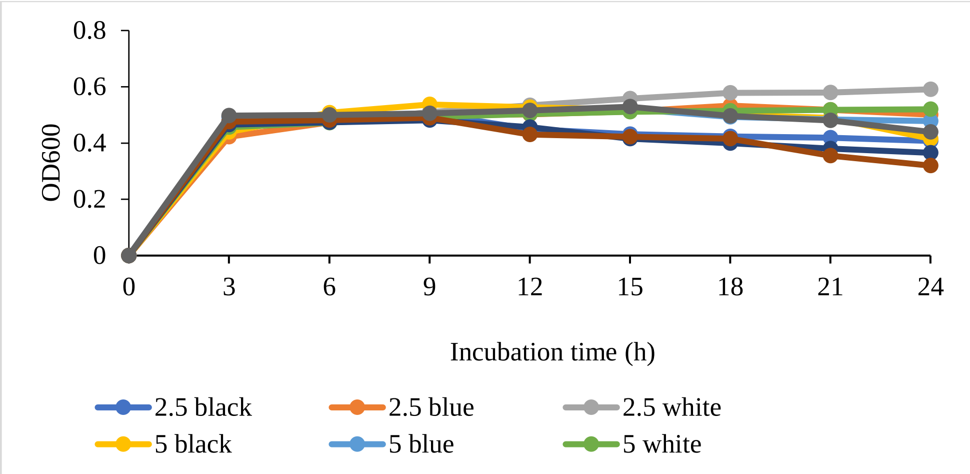

Figure 5: *Bacillus* sp. AP1 growth at MSM-face mask media under different NaCl concentrations (2.5, 5.0, 7.5%).

SEM Analysis

It is obvious in Figures 6-8 that morphological differences could be observed between the inner, middle and outer layers of the face masks. Typically, a disposable mask consists of three layers, i.e., an exterior hydrophobic non-woven layer, a middle melt-blown filter and an inner frontal non-woven layer [37]. All three layers of a 3-ply face mask underwent morphological changes when exposed to microorganisms. However, the mask central layer had undergone very few morphological changes as it still had melt-blown fiber. On the other hand, due to bacterial contact, the surface of the outer and inner layers was severely damaged with numerous scratches, fissures, and notches which could have been early signs of face mask biodegradation [38]. The morphological deterioration of the white KF94 face mask (Figure 8) was more significant than that of the blue surgical (Figure 7) and black duckbill (Figure 6) face masks respectively. This could have been affected by the content of the fabric, the type of yarn and construction of the face mask [39].

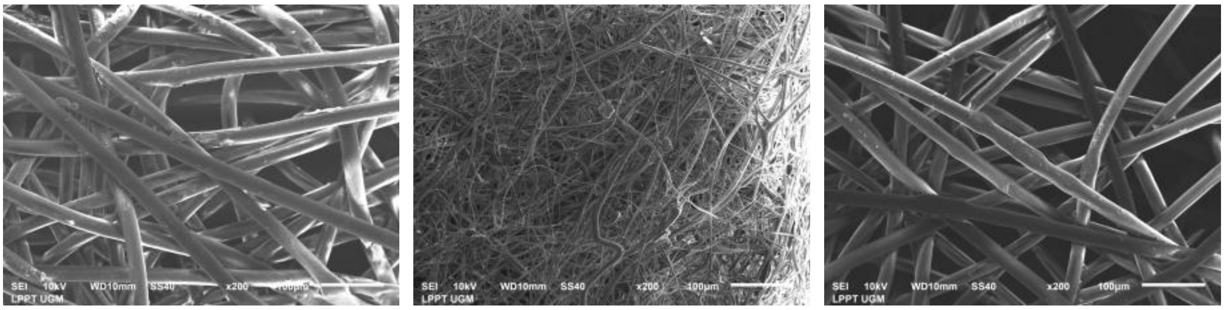

Figure 6: SEM analysis of black duckbill mask after contact with bacteria for 24h (A) outer layer (B) middle layer (C) inner layer.

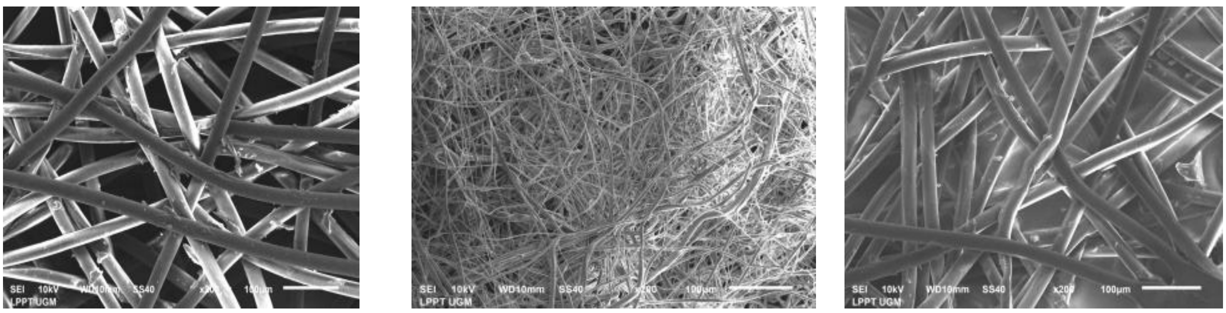

Figure 7: SEM analysis of blue surgical mask after contact with bacteria for 24h (A) outer layer (B) middle layer (C) inner layer.

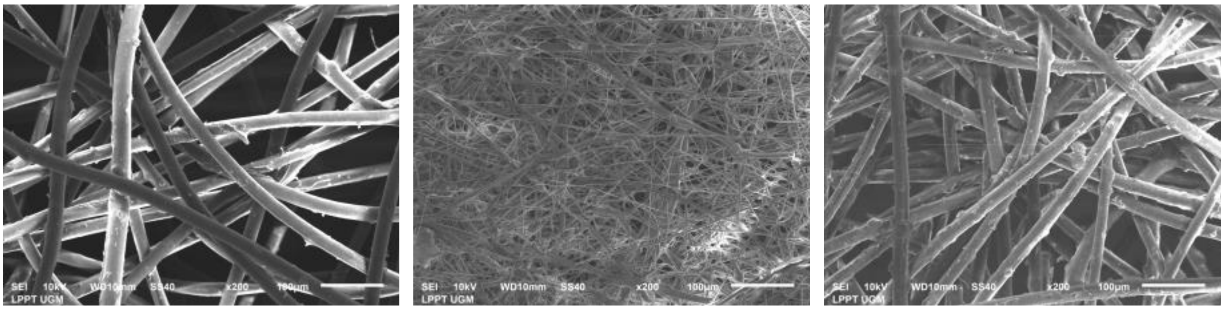

Figure 8: SEM analysis of white KF94 mask after contact with bacteria for 24h (A) outer layer (B) middle layer (C) inner layer.

Physical and Chemical Soil Properties

Indigenous face mask bacteria can originate from the surrounding environment. Adaptation and competition with other indigenous bacteria are often related to the environmental conditions around disposable face masks. According to Sun *et al.* bacterial species gradually adapts to its soil environment. Table 1 lists the soil physicochemical parameters surrounding face mask disposal sites.

Table 1: Soil physicochemical properties

Component	Value
Texture	
Sand (%)	88
Silt (%)	7
Clay (%)	5
pH	7.64
Salinity (µS/cm)	529
Moisture level (%)	0.11
C organic (mg/kg)	392.569
Organic matter (%)	1.17
Nitrogen (N) total (mg/kg)	205.112
Phosphor (P) (mg/kg)	303.364
Kalium (K) (mg/kg)	171.049
C/N ratio	1.9
Cation Exchange Capacity (CEC) cmol (+)/kg	0.40

Based on soil physicochemical studies, the soil is deficient in fertility and supports reduced bacterial development. The physical, physicochemical and biological soil properties require an understanding of soil texture [41]. The texture of the soil is determined to be sandy loam with 88% sand, 7% silt and 5% clay respectively. This soil texture is associated with bacterial diversity [42]. Reduced bacterial diversity in sand texture predominates [43]. Lesser clay content suggests insufficient organic matter [44]. Additionally, organic matter can affect ions adsorption and solubility [45]. The pH is a crucial component for bacteria and influences the biodegradability of face masks [46]. Since most bacteria are neutrophile, a soil pH of approximately 7 facilitates their growth. In addition to salinity, conductivity can be used to classify freshwater. Salinity influences the amount and diversity of bacteria, the activity of bacteria and the activity of enzymes is found to be the primary environmental factor that causes the bacterial community structural differences [47]. The ratio of carbon to nitrogen in the soil significantly affects the amount of accessible microbe-friendly nutrients. The low C/N ratio (1.9) induces bacterial community to use face masks as a nutrient source. The low CEC values with sand-based soils represent weaker soil capacity to absorb and exchange soluble cations [48].

Conclusion

Eight bacterial isolates were successfully isolated from three types of disposal face masks samples from Parangtritis Beach, Yogyakarta, Indonesia and were identified as *Bacillus* sp. (AP1-AP3), *Pseudomonas* sp. (AP4-AP5), and *Staphylococcus* sp. (AP6-AP8). The capacity of bacteria to grow on minimal media with face masks as the sole carbon source indicated their potential to degrade face masks. One of the isolates, i.e., *Bacillus* sp. (AP1) demonstrated the highest growth. However, the degradation of face masks by this bacteria was still limited to its degradation characteristics and related degradation enzymes. Further research was required to determine this bacteria's capacity to degrade face masks in various conditions.

Acknowledgements

The Ministry of Education, Culture, Research, and Technology of the Republic of Indonesia financed this research through *Penelitian Dasar Kompetitif Nasional* (PDKN) 2022. The authors acknowledged Universitas Negeri Yogyakarta's contribution to the completion of this study.

Conflict of Interest

The authors declare that they have no conflicts of interest.

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