FORMULATIONS OF LIQUID CARRIER MEDIA BASED ON NATURAL INGREDIENTS FOR HYDROLYTIC AND NITRIFYING BACTERIAL CONSORTIUM OF ORGANIC WASTE BIOREMEDIATION AGENT

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Abstract. The study aimed to develop a liquid carrier medium formulation composed of natural materials to support the growth and viability of hydrolytic and nitrifying bacterial consortia for organic waste bioremediation. The bacterial consortium consisted of 5 hydrolytic and 4 nitrifying isolates. The growth of the five hydrolytic bacterial isolates in growth media M1, M2, M3, M4, and M5 during the 30-day incubation period ranged between $1.03x10^9$ – $5.5x10^9$ CFU/mL, while the growth of the four nitrifying bacterial isolates in the five types of growth media ranged between $4.62x10^7$ – $2.91x10^9$ CFU/mL. The growth media M2, M1, and M3 resulted in an average total population of bacterial isolates of $2.53x10^9$, $2.41x10^9$, and $2.29x10^9$ CFU/mL, respectively. The total population of hydrolytic and nitrifying consortia in selected liquid carrier media (M1, M2, M3) decreased with increasing incubation time until 56 days. A significant decrease was found in the control of LB medium and liquid carrier medium M2, from 7.78 log CFU/mL to 5.11 log CFU/mL, and 8.20 log CFU/mL to 5.85 log CFU/mL, respectively. The total bacterial population in M3 and M1 on the 56th day of incubation showed a smaller decrease, from 7.23 and 7.91 log CFU/mL to 6.27 l and 6.58 log CFU/mL, respectively. The use of natural ingredients, such as banana stem and coconut water, in the M2 and M3 media was shown to support the viability of hydrolytic and nitrifying bacteria for 56 days of incubation.

Keywords: Banana stem, carrier media formulation, coconut water, hydrolytic bacteria, nitrifying bacteria

1. Introduction

The bacterial consortium comprising hydrolytic isolates LG73, LG101, LG113, SA126, and LG127, and nitrifying isolates BA26, BA38, SA14, and SA37, significantly reduced the C/N ratio and humic acid levels compared with a commercial inoculum during the degradation of organic waste [1]. Hydrolytic bacterial isolates were obtained from mangrove sediments at Logending Beach, Kebumen [2], and nitrifying bacterial isolates were obtained from fishpond sediments [3]. Further development of those bacterial isolates involves, for instance, creating a growth or carrier medium formulation to enhance their effectiveness as agents for organic waste biodegradation. Bacterial propagation using commercial synthetic media is expensive [4]; therefore, it is necessary to modify the composition of the carrier media by using economical natural materials.

The growth media used in industrial-scale bioremediation agents must maintain viability at high population densities. Formulating carrier media that are highly viable, inexpensive, and have a long shelf life is essential. Several natural ingredients have been shown to maintain the

viability of bacterial inocula, including coconut water [5], compost, sawdust, rice flour, rice bran, cow feces, sugar cane waste, and tofu waste [6]. The composition of laboratory media can be replaced by natural materials, which are more affordable and easier to obtain. Natural ingredients such as molasses, banana stems, and coconut water are known to contain high amounts of carbohydrates, proteins, and minerals. The nutrients contained in banana stems include: dry matter 87.7%, ash 25.12%, crude fat 14.23%, crude fiber 29.40%, crude protein 3%, including amino acids, ammonia nitrate, glycosides, containing N, glycolipids, vitamin B, nucleic acids, nitrogen-free extracts 28.15%, including carbohydrates, sugars, and starch [7]. The nutrients in banana stems include calcium (16%), potassium (23%), and phosphorus (32%) [8].

Molasses is a by-product of the sugarcane industry; the sugar content in molasses is quite adequate and ranges between 40-60% [9]. Sugarcane molasses also contains calcium, potassium, sodium, magnesium, sulfate, sulfite, silicate, chloride, as well as aconite, oxalic, malic, and citric acids [10]. Molasses also contains amino acids and minerals [11], which have great potential for utilization. Molasses contains active compounds such as sucrose (48.8%), fructose (8.45%), glucose (7.80%), as well as amino acids and polyphenols, including diosmin, which have functional benefits in the food and health sectors [12].

Coconut water is a clear liquid found in the endosperm (kernel) and is usually consumed fresh, especially when young [13]. Coconut water is the most potent organic material as a stimulant compared to molasses or bran. This is because coconut water contains glucose, fructose, sucrose, glutamic acid, and aspartic acid, which can stimulate phosphate-solubilizing bacterial activity [14].

The selection of natural ingredients must account for the nutritional requirements for bacterial growth. A medium formulation consisting of a mixture of several natural ingredients such as brown sugar (30 g/L), molasses (30 g/L), bean sprouts (60 g/L), corn starch (60 g/L), agar-agar (2 g/L), and fish meal or chick feed concentrate (10 g/L) is used as a carrier medium for the biofertilizer agent microbe inoculum [15]. The medium formulation can be modified to serve as a carrier for a consortium of hydrolytic and nitrifying bacteria for organic waste bioremediation by adding banana stem extract and coconut water as nutritional sources.

The addition of banana stem extract and coconut water as nutritional sources to a carrier medium formulation for a consortium of hydrolytic and nitrifying bacteria for organic waste bioremediation is valuable. An effective carrier medium should increase the viability or survival of microbes growing within it. Furthermore, the carrier medium can also enhance the biological activity of the inoculant by protecting the bacteria from biotic and abiotic stress [16]. The carrier medium, or any addition made after bacterial growth, should be capable of maintaining cell viability during storage [17].

This study aimed to identify the optimal natural liquid carrier media formulation for the growth of hydrolytic and nitrifying bacterial isolates and to assess the viability of a hydrolytic and nitrifying bacterial consortium for organic waste bioremediation in selected natural liquid carrier media.

2. Methods

2.1. Materials

Hydrolytic and nitrifying bacterial isolates are maintained in the Microbiology Laboratory, Faculty of Biology, University of Jenderal Soedirman, in nutrient broth supplemented with 20% glycerol. The materials used to prepare the formulation media composition were molasses, banana stems, coconut water, bean sprouts, brown sugar, corn starch, fish meal, and agar.

2.2. Preparation of liquid carrier media formulation with the addition of natural ingredient composition

Natural ingredients used as additional sources of carbohydrates, protein, and minerals in various liquid carrier media were banana stems and coconut water (Table 1). The basic ingredient composition for the liquid carrier media formulation of a medium 1 (M1) type is brown sugar (30 g/L), molasses (30 g/L), bean sprouts (60 g/L), corn starch (60 g/L), agar (2 g/L), and fish meal (10 g/L) [15]. Bean sprout extract was prepared by grinding the sprouts, adding 500 mL of distilled water, and collecting the extract. All ingredients were mixed, and distilled water was added to a volume of 600 mL. The mixture was boiled and filtered using a cloth. Then, 54 mL of the prepared medium was transferred into a 100 mL bottle and sterilized by autoclaving for 15 minutes at 121°C and 15 psi.

The composition of the liquid medium formulation ingredients for medium 2 (M2), medium 3 (M3), and medium 4 (M4) was a modification of medium 1 (M1) with variations in the concentration of nutrient sources of banana stems and coconut water, but without corn starch and agar (Table 1). The banana stem was extracted by cutting it into small pieces, mashing it, adding 500 mL of distilled water, stirring, and taking the extract. All ingredients were mixed, and distilled water was added to a volume of 600 mL. The entire mixture was boiled and filtered through a cloth. A total of 54 mL of medium was then transferred to a 100 mL volume bottle and sterilized by autoclaving for 15 minutes at 121°C and 15 psi. The compositions of the third, fourth, and fifth liquid carrier media were the same as that of the second medium, differing only in the concentrations of banana stem extract (300, 400, 500 g/L) and coconut water (25, 50, 75 mL/L) (Table 1).

Table 1. Composition of liquid carrier media formulation with the addition of natural ingredients

Material	Medium 1	Medium 2	Medium 3	Medium 4	Medium 5
Brown sugar (g)	30	30	30	30	30
Molasse (g)	30	30	30	30	30
Bean sprouts (g)	60	60	60	60	60
Banana stem (g)		60	300	400	500
Coconut water (mL)		100	25	50	75
Corn starch (g)	60				
Agar(g)	2				
Fish meal (g)	10	10	10	10	10
Aquadest (mL)	until 1000	until 1000	until 1000	until 1000	until 1000

2.3. Assay of liquid carrier media formulations on the growth of bacterial isolates, members of the consortium

Each isolate of hydrolytic bacteria (5 isolates) and nitrifying bacteria (4 isolates) was recultured on Luria Bertani (LB) Agar medium. Each isolate was measured for population density at 108 CFU/mL using the spectrophotometric method at 600 nm, followed by enumerationby the total plate count (TPC) method.

Assay the growth ability of each bacterial isolate in the first to fifth medium formulation was carried out by taking 10 mL of bacterial inoculum with a density of 10^8 CFU/mL into 90 mL of the first to fifth liquid media in an Erlenmeyer flask. The culture was then incubated on a shaker incubator at 30 °C for 30 days. Population growth of each bacterial isolate was measured on days 0, 15, and 30. A total of three liquid medium formulations that produced high population densities were used for further experiments (2.4).

2.4. Assay of selected liquid carrier media formulations on the viability of hydrolytic and nitrifying bacterial consortia

This experiment was conducted using a completely randomized design with four treatments: selected carrier media formulations and a control (LB medium). Each treatment was replicated three times. Data were analysed using analysis of variance (ANOVA) at the 5%

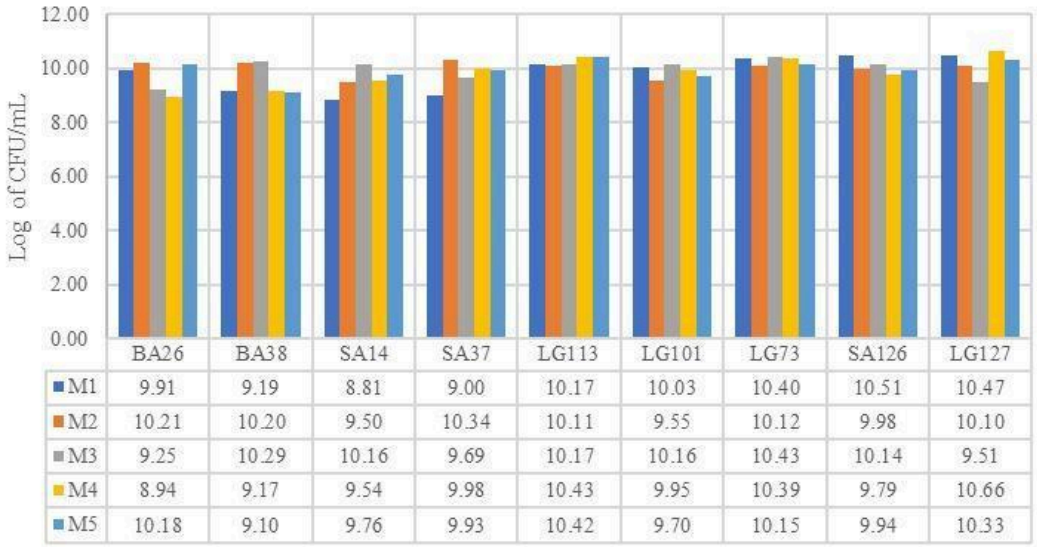
significance level. The analysis was continued with Duncan's multiple-range test (DMRT) at the P = 0.05 level.

The liquid carrier media used were the three best media resulting from experiment 2.3, and Luria Bertani Broth medium was used as a control medium. The volume of each liquid carrier media used in this experiment was 2.5 L. A total of 250 mL (10% v/v) of hydrolytic and nitrifying bacterial consortium inoculum was added to 2.25 L of each liquid carrier media type. Oxygen supply in the fermenter tank was provided by an aerator. The experiment was carried out for 56 days at room temperature. Measurements of total bacteria and media pH were carried out on days 0, 20, 40, and 56.

3. Results and Discussion

3.1. The growth of bacterial isolates in liquid carrier media formulation with the addition of natural ingredient composition

The study results showed that the three medium compositions, namely M2, M1, and M3, had the highest average total population among the 5 liquid carrier media tested (Figure 1). The average total bacterial population of the three medium compositions was $2.53x10^9$ CFU/mL, $2.41x10^9$ CFU/mL, and $2,29x10^9$ CFU/mL, respectively (Table 2). Medium M1 is a liquid carrier medium known as a liquid organic fertilizer containing the biofertilizer of Bacillus, Pseudomonas, and Streptomyces [15]. The ingredients of medium M1 are brown sugar, molasses, bean sprout extract, corn starch, agar, and fish meal. Meanwhile, medium M2 and M3 contain natural ingredients such as banana stem extract and coconut water, without the addition of corn starch and agar (Table 1).



	BA26	BA38	SA14	SA37	LG113	LG101	LG73	SA126	LG127
M1	9.91	9.19	8.81	9.00	10.17	10.03	10.40	10.51	10.47
M2	10.21	10.20	9.50	10.34	10.11	9.55	10.12	9.98	10.10
M3	9.25	10.29	10.16	9.69	10.17	10.16	10.43	10.14	9.51
M4	8.94	9.17	9.54	9.98	10.43	9.95	10.39	9.79	10.66
M5	10.18	9.10	9.76	9.93	10.42	9.70	10.15	9.94	10.33

Figure 1. Population density of each bacterial isolate (log CFU/mL) in 5 variations of liquid carrier media (M1, M2, M3, M4, M5) with an incubation time of 30 days.

The results showed that M2 medium supported the growth of hydrolytic and nitrifying bacteria better than M1 medium. This indicated that the addition of natural ingredients, such as banana stem extract and coconut water, as nutrient sources for the growth and reproduction of hydrolytic and nitrifying bacterial isolates was effective. Banana stem waste is a potential raw material for liquid organic fertilizer because it contains essential elements required by

microorganisms, such as nitrogen (N), phosphorus (P), and potassium (K) [8]. Meanwhile, coconut water contains several bioactive components, including proteins, amino acids, fatty acids, minerals, vitamins, and phenolic compounds. The bioactive components in coconut water are mainly antioxidants and arginine [18].

The results of pH measurements of the liquid carrier media for the hydrolytic and nitrifying bacterial consortium showed that pH decreased in all types of liquid carrier media after 30 days of incubation. The largest pH decrease was observed in medium M1, which contained brown sugar, molasses, bean sprouts, corn starch, agar-agar, and fish meal, with an initial pH of 7.18 and a final pH of 4.99. On the contrary, the average pH value of liquid carrier media containing coconut and banana stem extract (media M2, M3, M4, and M5) showed a lower pH decrease, with a final pH ranging from 5.21 to 6.21. These results indicated that the pH of the liquid carrier media containing natural ingredients of banana stem and coconut water supports the growth and development of hydrolytic and nitrifying bacterial isolates.

Table 2. Average total population density of each bacterial isolate at 30 days of incubation time

Type of media	Mean total bacterial population (log CFU/mL)	Mean total bacterial population (CFU/mL)
M4	9.1001±1,149	1.26×10^9
M5	9.2043±1,183	1.60×10^9
M3	9.3592±1,090	2.29×10^9
M1	9.3819±1,204	2.41×10^9
M2	9.4038±0,983	2.53×10^9

3.2. Assay results of the selected liquid carrier media formulation on the viability of hydrolytic and nitrifying bacterial consortia

The total bacterial population in the selected liquid carrier media (M1, M2, M3) and the control (K) decreased with increasing incubation time (Figure 2). A substantial decrease was observed in the control and M2 liquid carrier media treatments, from 7.78 log CFU/mL to 5.11 log CFU/mL and from 8.20 log CFU/mL to 5.85 log CFU/mL, respectively. In contrast, the decrease in the total bacterial population in liquid carrier media M3 and M1 was smaller, namely from 7.23 to 6.27 log CFU/mL and from 7.91 to 6.58 log CFU/mL, respectively.

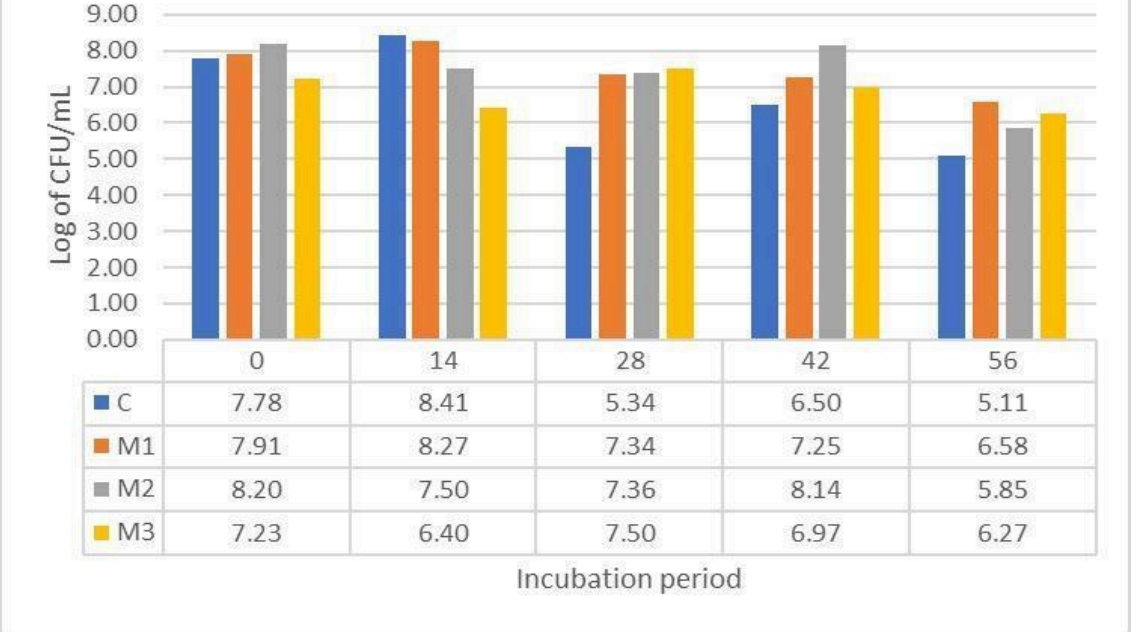


Figure 2. Total bacterial population in each carrier media formulation during the 56-day incubation period.

ANOVA results showed that the type of carrier media significantly affected the total bacterial population at the 5% significance level (Table 3). This was because each type of growth media contained a different composition.

Table 3. ANOVA of the effect of the type of liquid carrier media on the total bacterial population

Source of variance	df	Sum of squares	Mean squares	F calculation	F table (5%)	Significance
Treatment	3	3.154	1.051	6.602	4.76	0.025
Error	6	0.956	0.159			
Total	12	411.544				

The DMRT analysis showed that medium M1 had the highest average TPC (2.34x106 CFU/mL), followed by M3 (1.25x106 CFU/mL), M2 (6.76x105 CFU/mL), and the control (1.00x105 CFU/mL) after 56 days of incubation. Based on the average total bacterial population, medium M1 produced the highest population (6.37 log CFU/mL), although the increase was not significantly different from the M2 and M3 treatments and was significantly higher than the control (Table 4). These results indicated that the M1, M2, and M3 liquid carrier media better support the growth of hydrolytic and nitrifying bacteria in organic waste bioremediation than the control medium, LB.

According to [23], the cell viability of *Streptomyces* sp. strain ASR67 and the mixed culture of *Streptomyces* sp. strains ASR58 and ASR67 was higher in sugar potato liquid media compared to skim molasses and rice bran liquid media. The cell viability of strain ASR 58 was $5.3x10^7$ CFU/mL, ASR 67 was $3.8x10^7$ CFU/mL, and the combination of both was $4.3x10^7$ CFU/mL. The strains were best maintained in talc powder carrier media for 10 weeks of storage. Meanwhile, the bacterial population of biofertilizer *Burkholderia nodosa* in liquid coconut medium after 60 days of incubation was 4x108 CFU/mL [4].

Table 4. Results of DMRT analysis of media type treatment on average bacterial viability at 56 days of incubation time

Type of media	Mean total bacterial population (log CFU/mL)
Control	5.0000 a
M2	5.8300 b
M3	6.0967 b
M1	6.3700 b

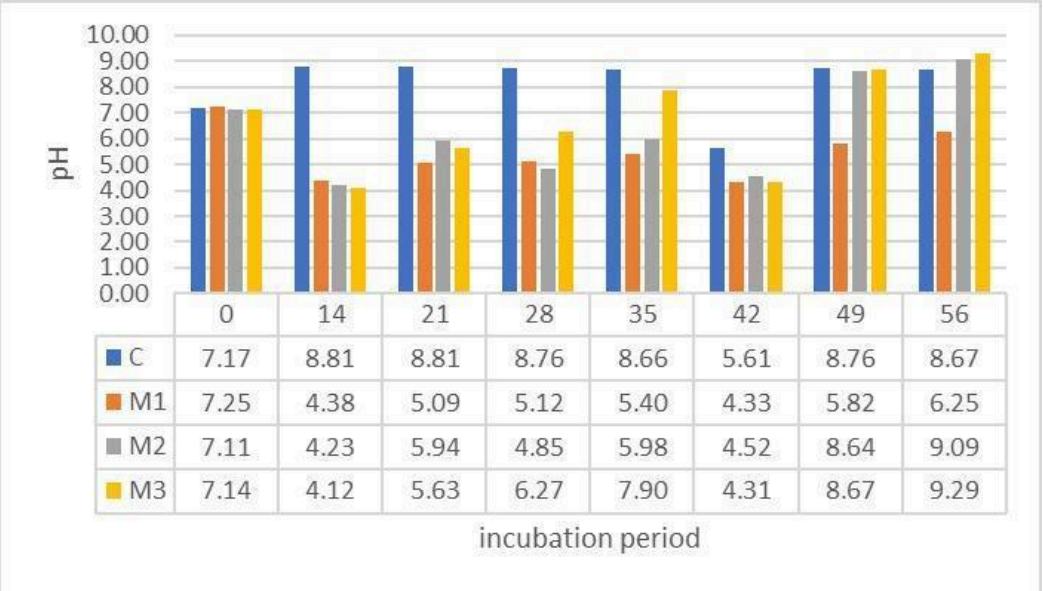
Note: Different letters after the numbers indicate that the treatments differ significantly at $P < 0.05$.

The total number of bacterial populations in the liquid carrier medium containing natural ingredients such as molasses, banana stem extract, and coconut water was higher than that in the commercial medium Luria Bertani during 56 days of incubation. This showed that the nutritional content in natural ingredients really supports the growth and development of hydrolytic and nitrifying bacteria. Molasses contains non-carbohydrate components, including protein (4.38%) and ash (6.20%), indicating the presence of nitrogen and minerals beneficial to microbial growth [19]. In addition, molasses contains essential amino acids, such as glutamic acid, glycine, proline, tyrosine, and valine, at low levels, which also support biological activity during fermentation.

Formulation of carrier media derived from agricultural waste (molasses, coconut water, and bran) can increase the viability of phosphate-solubilizing bacteria (PSO), growth, and soybean plant yield (Glycine max L.) on Inceptisols[14]. Coconut water can significantly increase the number and weight of seeds per soybean plant, with yield increases of 41.176% and 18.823%, respectively. Coconut water is the most potent organic material as a stimulant compared to molasses or rice bran. This is because coconut water contains glucose, fructose, sucrose, glutamic acid, and aspartic acid, which are implicated in stimulating PSO activity.

The use of natural agricultural waste materials, such as banana stem extract and coconut water, has been shown to support the viability of hydrolytic and nitrifying bacteria of organic waste bioremediation agents during 56 days of incubation. This indicated that the nutrient availability in the natural liquid carrier media supports the growth and reproduction of the hydrolytic and nitrifying bacterial isolates. Banana stem waste is a natural material that can be used to manufacture liquid fertilizer [24] and liquid organic fertilizer (POC) [25].

The results of pH measurements of the liquid carrier media for hydrolytic and nitrifying bacteria over 56 days of incubation showed that the pH in the control treatment (LB medium) increased with incubation time (Figure 3). On the other hand, the pH value of liquid carrier media M1, M2, and M3 decreased during incubation from day 14 to day 42. This occurred due to the high activity of hydrolytic bacteria degrading carbohydrates in the medium, resulting in a slightly acidic pH. The increase in pH of the liquid carrier media from day 49 to day 56 of incubation was caused by the activity of the hydrolytic bacteria group in breaking down proteins into amino acids. Meanwhile, a decrease in pH can be caused by the activity of nitrifying bacteria, in which ammonium is oxidized to nitrite, producing NO_2^- and $H^+ + H_2O$. The resulting H^+ ions decrease pH because they are acidic [20]. A decrease in pH can also result from the presence of nutrients in sufficient quantities, such that optimal bacterial activity produces organic acids that reduce acidity [21]. Changes in the pH of a growth medium can be caused by microbial activity and by other factors, such as the medium type, nutrient availability, and environmental conditions [22].



	0	14	21	28	35	42	49	56
C	7.17	8.81	8.81	8.76	8.66	5.61	8.76	8.67
M1	7.25	4.38	5.09	5.12	5.40	4.33	5.82	6.25
M2	7.11	4.23	5.94	4.85	5.98	4.52	8.64	9.09
M3	7.14	4.12	5.63	6.27	7.90	4.31	8.67	9.29

Figure 3. The pH value of the liquid carrier media during the incubation period of 56 days

4. Conclusion

It can be concluded that liquid carrier media formulations M1, M2, and M3 containing natural ingredients can support the growth of hydrolytic and nitrifying bacterial isolates. The liquid carrier media M1, M2, and M3, containing natural ingredients, were able to maintain the viability of the consortium of hydrolytic and nitrifying bacteria used in organic waste bioremediation.

5. Acknowledgement

The author would like to thank the Institute for Research and Community Service of Jenderal Soedirman University for the “Professor Facilitation Research” funding scheme 2025.

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