Evaluation of Bacillus Brevis in Microbial-Induced Calcite Precipitation of Threshold Friction Velocity and Crust Thickness for Wind Erosion Control of Aeolian Soil

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ABSTRACT

An eco-friendly method of soil improvement known as Microbial Induced Calcite Precipitation (MICP) has received significant recognition in the past decade. This study presents a report on the capability of MICP in modifying the threshold friction velocity (TFV) and soil crust thickness of aeolian soil bio-treated at various suspension densities of a ureolytic microorganisms - Bacillus brevis (B. brevis) and cementation reagent of varying concentration. The B. brevis suspension densities and cementation reagents used to trigger the MICP process are 0, 0.5, 2.0, 4.0, 6.0 and 8.0 McFarland standards (i.e., 0, 1.50×10^8, 6.0×10^8, 1.20×10^9, 1.80×10^9 and 2.40×10^9 cells/ml and 0.25, 0.5, 0.75 and 1.0 M, respectively. The results at various B. brevis suspension densities and cementation reagent concentration indicated improvement in threshold friction velocity (TFV) and soil crust thickness of aeolian soil bio-treated. Bio-treatment of aeolian with 6.0×10^8 cells/ml - 0.75 M mix ratio improved the critical threshold friction velocity (TFV) of natural aeolian soil from 13.6 m/s to 18.2 m/s similarly, the soil crust thickness of the bio-treated aeolian soil specimens with 6×10^8 cells/ml – 0.75 M and subjected to the maximum wind speed load of 20 m/s improved from the natural powdery form with no crust thickness to 90 mm. these parameters contributed significantly in improving wind-induced resistance.

Keywords: Aeolian soil, Bacillus brevis, Microbial-induced calcite precipitate, threshold velocity (TFV), soil crust

INTRODUCTION

Crusts on soil surfaces are well recognized for wind erosion control in regions with either scarce or no vegetative cover (Duniway *et al.,* 2019; Fick *et al.,* 2020) and mitigation of dust emission from mine tailings and other sources (Gil-Loaiza *et al.,* 2018; Nikseresht *et al.,* 2020). Due to the growth of macrobiotic calcium carbonates, and clay minerals in soil, biotic crusts formed on the soil surface in nature (Belnap and Büdel, 2016; Dai *et al.,* 2016).The threshold friction velocity (TFV) play vital role in identifying the rate by which the soil sediments will start eroding by the wind (Gao *et al.,* 2023), several researches have been conducted to determine the TFV of various soil samples (Van Pelt *et al.,* 2017; Von Holdt *et al.,* 2019; Gao et al., 2023; Fattahi *et al.,* 2020a). Soil erosion by the wind majorly in arid and semi-arid regions is a threat to the sustainability of the land and quality of life for rural and urban communities. Wind erosion depends on how easily soil particles can be taken from the land surface and is thus closely related to penetration resistance, which reflects the soil crusts hardness and bonding strength between particles (Hao *et al.,* 2021). This paper

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presents the evaluation of TFV and crust thickness of Aeolian soil bio-treated with b. brevis at various suspension densities and cementation reagent of varying concentration using wind tunnel chamber.

Microbial Induced Calcite Precipitation (MICP) is a new soil treatment approach that emerged recently for developing soil erodibility resistance that provide sufficient resistance to wind erosion (Neimi *et al.,* 2023; Gao *et al.*, 2023). Microbiology, geochemistry, and civil engineering are the inter-disciplines by which this new opportunity has emerged. This field is new and exciting opportunities lie ahead to the researchers for the field to be fully understood (Minyong *et al.,* 2020; Sani and Bala, 2021). The technique is quite sustainable and environmentally friendly and is used to transform soil behaviour like TFV, crust thickness, strength and toughness (DeJong *et al.,* 2010; Devrani *et al.,* 2021; Abubakar, 2023). The method is a biologically natural method. Urea hydrolyzing bacteria are mostly used in most applications of MICP. The technique is very simple, long-lived, low cost as well as efficient and there is no excess proton production (Whiffin *et al.,* 2007; Armstrong *et al.,* 2021). This allows the microbial bio-cementation to be more ecologically friendly in improving soil crust thickness and TFV for wind induced erosion control. The approach utilized genetically modified bacteria so as to improve the efficiency of carbon dioxide (CO_2) conversion into into calcite to limit its emission to the minimal level. The calcite produced during MICP acts as a carbon sink, storing CO_2 in a stable solid form.

MATERIAL AND METHODS

Materials

Soil sample

The aeolian soil used in this research was collected from Wudil (11^{o}47’39.2712” N and 8^{o}50’20.5152” E) Local Government Area in Kano state, Nigeria. The soil was collected from a depth of 15 cm using a sterile tool. The soil samples were transferred to a clean polythene bag and transported to the Soil Mechanics Research Laboratory of the Ahmadu Bello University, Zaria, Kaduna State, Nigeria.

Microorganism

The microbe used in the study is B. *brevis*, which is normally found in soils. The urease positive microbes is rod-shaped, spore-forming and Gram-positive; it was cultured from the soil sample.

Cementation reagent

The cementation reagent, which has been used in multiple studies, is made up of 20 g of urea, 10 g of NH_4Cl, 3 g of nutritional broth, 2 g of CaCl4, and 2 g of $NaHCO_3$ per liter of distilled water (e.g., Stocks-Fischer *et al.,* 1999; DeJong *et al.,* 2006; Al Qabany *et al.*, 2012; Park *et al.,* 2014; Feng and Montoya, 2016; Tirkolaei and Bilsel, 2015). In all the studies mentioned, 3 gL of nutrient broth was added to a cementation reagent because, it was the most effective amount for bacteria survival (Sharma and Ramkrishnan, 2016). However, the arrangement was varied to obtain dissimilar concentrations (i.e., 0.25, 0.5, 0.75 and 1.0 M).

Methods

Seclusion of *B. brevis*

The tyrosine selection method, as outlined by Edwards and Seddon (2000), was used to extract *Brevis* from soil. Each sample was treated with 10g of soil and 90mL of distilled water. The samples were then incubated on a rotatory shaker (B) at 28^{o}C for 45 minutes, at 250 rpm (Bran Scientific and Instrument Company, England). After autoclaving at 121°C for 15 minutes in a 50 mL Erlenmeyer flask, 2 mL of the broth culture was added to 20 mL of sterile Tyrosine broth that contained (g/l) in distilled water: 6.5 nutrient broth and 5.0 tyrosine. The mixture was placed on a rotatory shaker set to 250 rpm and incubated for four hours at 28^{o}C. Following incubation, 5 mL aliquots of each culture were heated to 80 °C for three minutes in a hot water bath. Next, 0.1 mL of tyrosine agar (containing 6.5 g/L of nutritional broth, 5 g/L of tyrosine, and 15 g/L of agar)

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was spread out and incubated for 24 hours at 28ºC. Bacterial colonies on the plates were visually inspected following a 24-hour incubation period. Colonies exhibiting typical B morphological traits, such as light brown color, non-spreading, serrated edges, and clear halos of *brevis,* on Tyrosine agar, were sub-cultured.

Identification of *B. brevis*

An assumption-based characterization of the *B. brevis* was made using the biochemical and cultural traits of the isolates. *Brevis* was first verified using the retention factor (RF) on the thin layer chromatography plate in comparison to the standard (Gramicidin) (Cowan and Steel, 2003; Bergey, 2004).

Morphological Characterization

The pure isolates were Gram stained and the shape and arrangement of the cells was examined. Also, endospore staining was carried out to determine spore formation by the isolates.

Confirmation of *B. brevis* using Chromatography of Ethanolic Extracts

The chromatography analysis was carried out according to method described by Edwards and Seddon (2000) with some modifications. Five-day-old tryptic soya broth cultures (20 mL broth in 100 mL conical flask) were used to prepare ethanol extracts of the Bacillaceae species, which were then incubated at 28 ºC and 150 rpm. The pellet was re-suspended in 2 mL of ethanol after the broth cultures were centrifuged for 10 minutes at 3000 g, with the supernatant fluid extracted. On a rotatory shaker (B), the ethanol suspensions were combined for one hour. Prior to centrifugation at 3000 g in 10 min, the Bran Scientific and Instrument Company, located in England, was consulted.

The ethanolic extract was represented by the fluid that was removed as the supernatant. On a 10 x 10 cm sheet of paper chromatography plate, the extracts and antibiotic standard (1 mg/ml in ethanol for gramicidin using Neomycin antibiotic) were spotted using capillary tubes 1.5 cm above the baseline. Butanol: acetic acid: water (80:13:7) was the solvent utilized.

The paper was dried in a fume cupboard once the solvent reached the top of it and the solvent front was marked. After being allowed to air dry in a fume cupboard, the paper was sprayed with five percent w/v ninhydrin and baked for ten minutes at 80 degrees Celsius. The ninhydrin products' RF value was noted.

Methods

The wind erosion test using wind tunnel was conducted to simulate firmness of the outward soil cover induced due to MICP for minimizing the wind-induced erosion.

The weighed soil samples were placed on pans, prepared and treated in triplicates for each sample bio-treated. The treatment solutions were injected into the soil sample with the aid of syringe. After the treatment the samples were left for a period of 24 hours for proper hydration to take place before the introduction of wind load to the bio-treated specimens. In each test, the cumulative soil mass loss were obtained by measuring the soil weight in the pans before and after wind load application. During the test, the soil samples were subjected to a wind loads of 18, 22, 26, 30, 36, 42 and 46 m/s for a period of 60 seconds respectively.

The prepared soil sample was placed in the opening created in the floor of the wind tunnel. The wind tunnel was covered to maintain consistent lighting, and a variable brightness lighting system with all instrumentation in place. The wind tunnel was turned on to start the experiment.

The weighed soil samples were placed on pans, prepared and treated in triplicates for each sample bio-treated. The treatment solutions were injected into the soil sample with the aid of syringe. After the treatment the samples were left for a period of 24 hours for proper hydration to take place before the introduction of wind load to the bio-treated specimens. In each test, the cumulative soil mass loss were obtained by measuring the soil weight in the pans before and after wind load application. During the test, the soil samples were subjected to a wind loads of 18, 22, 26, 30, 36, 42 and 46 m/s for a period of 60 seconds respectively.

ISSN No. 2454-6194 | DOI: 10.51584/IJRIAS |Volume X Issue I January 2025

The sediment flux (qs) is the rate of sediment particles being eroded through a specific area over a particular time. It was calculated using equation (1).

Sediment flux, qs (kg/m^{2}s) = $[soil\ loss\ x\ (A\ X\ T)]$ (1)

Velocity, V = $\sqrt{2pwgHa/pa}$ (2)

Where, A = is the area and T = is time taken for the wind load, p = water pressure, g = acceleration due to gravity, Ha = height of the specimen, pa = atmospheric pressure

After bio-treating and mixing, the soil samples for the various microbial suspension densities, were air-dried before being crushed and passed through BS No. 4 sieve (4.75 mm opening) for use in the wind erosion testing. The weighed soil samples were treated in the circular metallic pan and left for 24 hours prior to wind erosion test.

RESULTS AND DISCUSSION

Index properties of the natural aeolian soil

The natural soil had a moisture content of 27.0 % as at the time of sampling in July 2021, during the rainy season. The physical properties of the soil sample are summarized in Table 1. The particle size distribution curve is presented in Figure 1. The soil is brownish in colour; it is non-plastic in nature and has 3.0 % fines passing through sieve No. 200. It is classified as A-3(0) soil group using the AASHTO system of soil grouping (AASHTO, 1986) and sandy silt (SM) by the Unified Soil Classification System (ASTM, 1992). Generally, in their natural state aeolian soils are loose materials and lack cohesiveness which tends to affect their strength and makes them unsuitable for most engineering projects such as pavement construction and building foundations. Based on the index properties of the soil stated above it requires improvement to be used in engineering works.

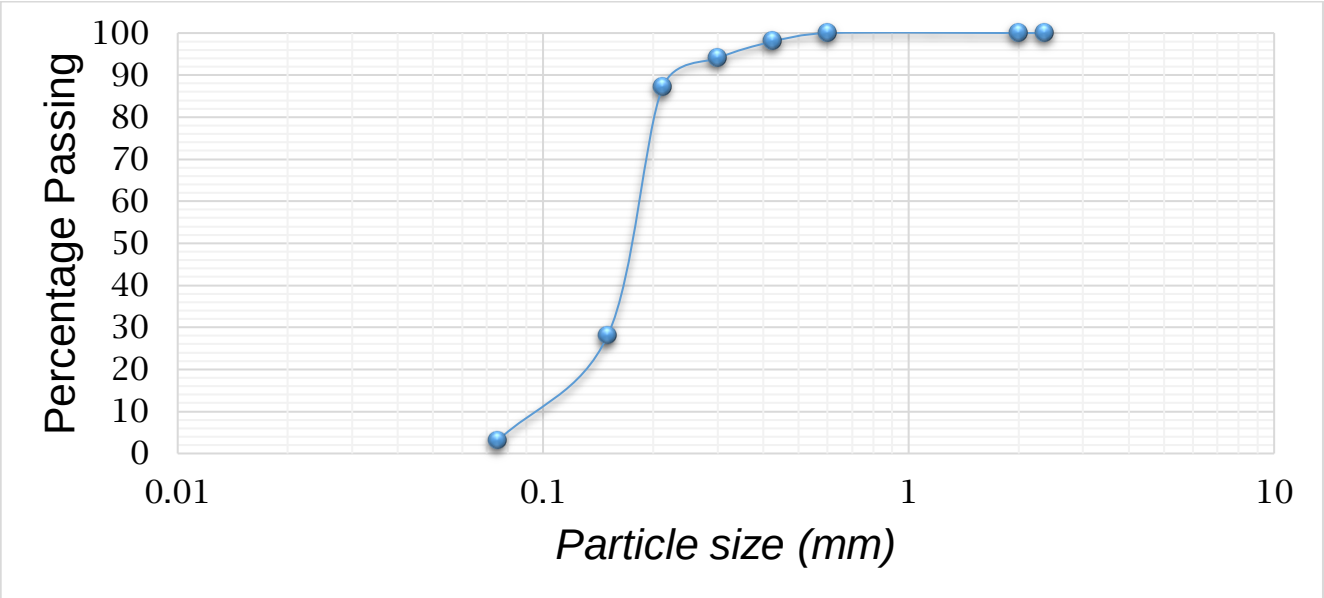


Figure 1: Particle size distribution curve for the natural aeolian soil.

Table 1: Properties of the natural aeolian soil used in the study

Property	Quantity
Percentage passing No. 200 sieve	3.0
Natural Moisture Content, %	27.0
Liquid Limit, %	-
Plastic Limit, %	Non-plastic

ISSN No. 2454-6194 | DOI: 10.51584/IJRIAS |Volume X Issue I January 2025

Plasticity Index, %	Non-plastic
Linear Shrinkage, %	-
Specific Gravity	2.63
AASHTO Classification	A-3(0)
USCS	SP-SM
Maximum Dry Density, Mg/m^3	1.62
British Standard light	1.63
West African Standard	1.72
British Standard heavy	
Optimum Moisture Content, %	
British Standard light	2.0
West African Standard	1.90
British Standard heavy	1.86
Colour	Brown

Threshold friction velocity (TFV) and soil crust thickness

This test was conducted at laboratory temperature of 25ºC, pressure of 89 kN/m^2 and air density of 1.22 g/m^3 for a period of 60 seconds (naturally, wind leading to significant erosion are often short-lived gusts or sustained winds of limited duration, the 60-seconds test duration can simulate these realistic conditions without requiring extended testing period) for all the wind velocities considered respectively.

Effects of B. *brevis* on threshold friction velocity (TFV)

The variation of TFV of the treated aeolian soil with *B. brevis* cells/ml and cementation solution at higher speed of 20 m/s are as shown in figure 2.

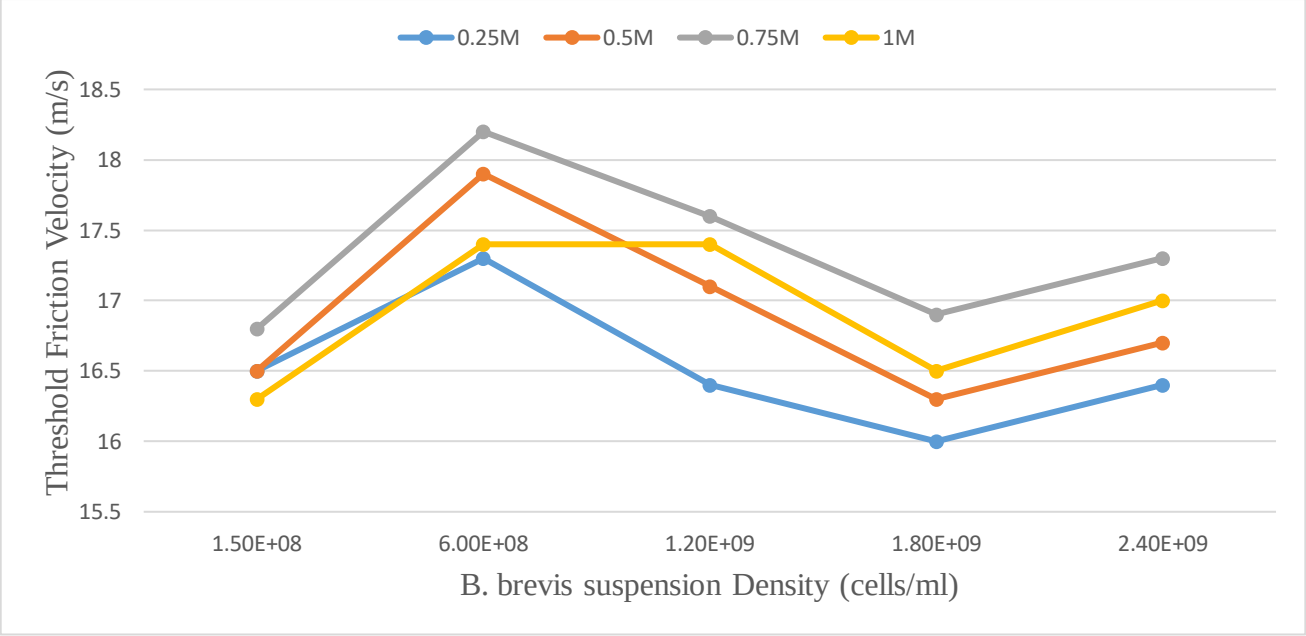
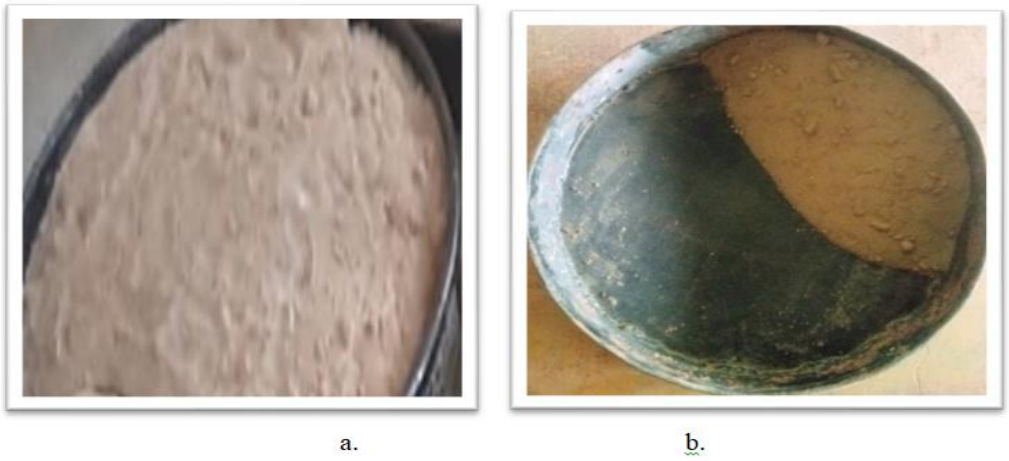


Figure 2: Variation of the TFV of the aeolian soil with *B. brevis* suspension density and cementation reagent treatment at 20 m/s wind speed

The TFV recorded for the natural soil samples, bio-treated soil samples are presented in plate I. The wind speeds were increased gradually during the testing until the soil surface experienced sustained erosion and was reported as Threshold friction velocity (TFV) of soil sample. It was observed from the results that the concentration of cementation solution had a significant influence on the threshold friction velocity. The TFV values increased initially until it reached a peak point of 18.2 m/s at 6.0 x 10^8 cells/ml; it then decreased thereafter until it reached 1.8 x 10^8 cells/ml and then increased slightly. The natural soil with no treatment encountered a TFV value of 13.6 m/s. Similarly the peak TFV value of 18.2 m/s was recorded for the sample

ISSN No. 2454-6194 | DOI: 10.51584/IJRIAS |Volume X Issue I January 2025

bio-treated at 6.0 x 10^8 cells/ml - 0.75M. It was also observed that the TFV values decreased for the bio-treated sample in comparison to the natural soil; this improvement in the TFV could probably be due to the formation of calcite bonds between the soils particles. This calcite formation held the soil particles together and led to an increase in resistance against the increase in wind speeds. Tian *et al*. (2018) reported an increase in the TFV from 5.73 m/s for untreated soil to 16 m/s after 3 doses of 0.5M treatment solution. Zomorodian *et al*. (2019) also found that soil treated with 0.5M cementation reagent solution experience no erosion with increasing wind speeds up to 20 m/s. The results obtained in the current study revealed the potential of the MICP treated soil against the wind speeds of 20 m/s treated at 6 x10^8 cells/ml – 0.75 M respectively.



a. b.

Plate I: Natural soil sample (a) before and (b) after experiencing wind load.

Effect of B. *brevis* on the soil crust thickness moulded

Plate III shows the soil sample with no crust (Plate II a) while bio-treated soil sample with crust thickness (Plate II b) prior to wind load application also, Plate IV shows the soil sample crust thickness of bio-treated soil sample treated for higher wind speed considered (20 m/s) at 6 x 10^8 cells/ml – 0.75 M. The crust was formed for the bio-treated soil compared to the natural one that is with little or no crust (powdery form). The soil crust thickness of treated soil specimens was measured after being subjected to the maximum wind speed load of 20 m/s at 6 x 10^8 cells/ml – 0.75 M for a period of 60 seconds. The soil crust thickness obtained was 90 mm. according to Fattahi *et al*. (2020a, b) crusts thickness formed above 15 mm thick, can crust provide significant protection against wind erosion and are often the result of sustained application of MICP techniques.

a. b.

Plate II: Crust thickness: (a) natural with no crust (powdery form) (b) bio-treated soil with crust thickness formed before wind-induced load

Previous studies have reported that the presence of calcium carbonate enhanced the crust formation in sandy soils (Devrani *et al*., 2021). Furthermore, the increase in the calcium carbonate precipitation with the increase in the b. *brevis* suspension density and cementation solution might probably have triggered the improvement in

ISSN No. 2454-6194 | DOI: 10.51584/IJRIAS |Volume X Issue I January 2025

the soil crust thickness formed. It was reported by Devrani *et al*., (2021) that bio-cemented crust formation by *Bacillus megaterium* provided considerable protection against wind erosion by diminishing wind erodibility indicators by two to four orders of magnitude. However, the presence of saltating grains could reduce the erodibility indicators only by one order of magnitude (Devrani *et al*., 2021).

Plate IV: Soil crust thickness of bio-treated aeolian soil sample treated for higher wind speed of 20 m/s at 6 x 10^8 cells/ml – 0.75 M.

CONCLUSION

From the results recorded in the study, the following conclusions can be made:

1. The critical threshold friction velocity (TFV) of the natural soil was 13.6 m/s while 18.2 m/s was recorded for bio-treated soil sample at 6.0 x 10^8cells/ml - 0.75M
2. The soil crust thickness of treated aeolian soil specimens was measured after being subjected to the maximum wind speed load of 20 m/s at 6 x 10^8 cells/ml – 0.75 M. The aeolian soil crust thickness 90 mm was recorded for the bio-treated soil sample while for the natural it's in powdery form with no crust thickness.

RECOMMENDATIONS

1. Treatment of Aeolian soil with 6.0 × 10^8 cells/ml - 0.75 M generated a TFV of 18.2 m/s while for the natural soil 13.6 m/s was recorded. Thus improved its Threshold Friction Velocity (TFV).
2. Aeolian soil bio-treated with B. *brevis* 6.0 × 10^8 cells/ml - 0.75 M developed a crust thickness of 90 mm from its natural powdery form, thus significantly improved residual crust thickness after erosion testing.

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