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Incorporation of Bokashi Fermented Leaves (BFL) to Improve the Algal Growth on Concrete Surface †

Abdul Mannan Yousfani [1,*], Tomohisa Kamada [2], Toshiharu Kishi [2] and Farhan Ahmed Shaikh [1]

[1] Department of Civil Engineering, Thar Institute of Engineering, Sciences and Technology, NED University of Engineering and Technology, Police Road, Mithi 69230, Pakistan
[2] Institute of Industrial Science, The University of Tokyo, 4-6-1 Komaba Meguro-ku, Tokyo 153-8505, Japan
* Correspondence: abdulmannanyousfani@neduet.edu.pk
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Abstract: Through the bokashi process, fermented leaves were incorporated as a partial alternative to sand in concrete to produce a concrete type that could be a prospective artificial reef material. The specimens were checked in small pools where algae—Chlorella vulgaris—was added and checked for growth. The results showed that the concrete with 20% bokashi fermented leaves (BFL) by weight of cement had 40 times higher algal coverage on the surface by the end of 35 days. Concrete with 2% bokashi fermented leaves (BFL), however, tended to increase the algal coverage by only 3 times.

Keywords: artificial reefs; bokashi fermented leaves; amino acids; carbo hydrates; adsorption; nutrients; algal growth on concrete; environment friendly concrete

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1. Introduction

The first artificial reef was probably accidentally created sometime in 1500s according to evidence found in the Mediterranean Sea. The rocks that were used to anchor the tuna fishing nets were left on the seabed at the end of every fishing season which, overtime, accumulated and formed a new rocky habitat [1]. The idea of a modern-day artificial reef first originated in Japan in the late 18th century when fishermen noticed a higher fishing yield around ship wrecks and later made their own wooden structures weighed with sand bags and sunk down in the sea at a depth of around 36 m [2].

Fast forward to today: many artificial reef materials have been used. However, concrete stands out, as it can serve most of the functions that an artificial reef is supposed to. It is not only physically stable in the underwater environment but also manifests little-to-no chemical degradation [3–5]. Moreover, concrete has proven to support a variety of biota on its surface. In fact, many studies have shown a higher coverage area and/or a diverse biota attachment on concrete reef surfaces compared with other materials such as wood, rubber, PVC, steel and scrap tires [6–9].

What makes concrete the most compatible material is the usage of Portland cement. Lime—a major raw material used to produce Portland cement—is a component of limestone, which is primarily made up of calcium carbonate ($CaCO_3$) that, interestingly, is also the substance that comprises coral reefs [3]. Another factor in the concrete, however, leads to problems. The concrete surface tends to have a high pH value from around 10 to 11 due to portlandite—calcium hydroxide (CaOH). This is significantly higher than the pH of seawater, which ranges in between 7.5 and 8.5. A higher pH value can make the surface of concrete toxic for some benthic organisms [3,10].

Researchers and the scientific community have been dealing with this issue in a very interesting manner. The usage of pozzolana and other cement additives can help reduce the pH of the surface of concrete [3]. Huang et al. [10], for example, used steel slag and blast furnace slag to reduce the production of portlandite (CaOH) and therefore

avoid the attachment of unwanted organisms such as barnacles. Alternatively, EConcrete Technologies, founded by two ecologists in Israel, use a mix of pozzolana and introduce a complex surface to reduce the pH and improve the attachment of benthic organisms [11].

Hence, most work on artificial concrete reefs has been limited to reducing the pH and playing with the surface of concrete. However, a few researchers have recently taken this work to a new level and started introducing nutrients such as amino acids inside the concrete, which might work as an attractant for benthic organisms [12,13]. In another study, Dennis et al. [14] used hemp fiber as a partial alternative to aggregate along with a ground granulated blast furnace slag (GGBS) as a partial replacement of cement, showing higher algal concentrations and mean live covers compared with normal concrete and other concrete types without hemp fiber. Although the researchers did not mention nutrients leaching out of the concrete, a similar phenomenon to that observed by Mohamad et al. [13] could be assumed in this study, which may have resulted in higher algal growth.

The authors of the presented study propose a sustainable nutrient technology—bokashi fermented leaves (BFL)—and evaluate its effects on the algal growth on a concrete surface.

2. Materials and Methods

2.1. Bokashi Fermented Leaves

Leaves are a good source of nutrients such as amino acids, vitamins, and minerals, and they have therefore been extensively used for agricultural purposes such as leaf mulch. They improve biological structures and increase the carbon content of soil [15–17]. However, when incorporated into waterbodies, they can be a bit tricky. When used without fermentation as a nutrient source, some kinds of leaves have shown lower growth and inhibitory effects for fishes and algae, respectively [18–21].

Bokashi—the Japanese word for fermented materials—is a set of effective microbes such as lactic acid bacteria, photosynthetic bacteria, actinomycetes, yeast and fungi that feed on the material in anaerobic environments to enact the fermentation. Here, effective microbes were locally obtained using an online shopping website—Rakuten. The effective microbes were prepared on rice bran and packaged by a Japanese company called EM Seikatsu, Nagoya, Japan.

Fallen leaves, regardless of their origin, were collected from the premises of the Institute of Industrial Science, The University of Tokyo. Leaves were then roughly checked, and any foreign material was removed. Effective microbes and the leaves were then placed into the anaerobic containers for 15 to 30 days. A white foamy structure (see Figure 1) appearing on the leaves manifested the completion of fermentation.



Figure 1. Fermentation of leaves.

Fermented leaves were then dried and chopped into a finer size so they could be used as a partial fine aggregate material. The physical properties and chemical composition of the fermented leaves were checked using appropriate methods such as NC analysis and WD-XRF; the results are shown in Tables 1–3.

Table 1. Physical properties of bokashi fermented leaves.

Bulk Specific Gravity	0.282 g/cm^3
Bulk SSD Specific Gravity	0.718 g/cm^3
Water Absorption	150%
Fineness Modulus	3.64

Table 2. Nitrogen and carbon contents of bokashi fermented leaves.

Sample 1	
Total Carbon	40.87%
Total Nitrogen	1.35%
Sample 2	
Total Carbon	47.91%
Total Nitrogen	1.31%

Table 3. Elemental composition of fermented leaves using WD-XRF.

S. No.	Element	Analytical Value (%)	Analysis Line	X-ray Intensity
1	Magnesium (Mg)	1.34	Mg-KA	0.2904
2	Aluminum (Al)	1.35	Al-KA	0.8752
3	Silicon (Si)	21.7	Si-KA	13.2812
4	Phosphorus (P)	4.11	P-KA	5.1787
5	Sulphur (S)	1.47	S-KA	1.4525
6	Chlorine (Cl)	0.618	Cl-KA	0.1376
7	Potassium (K)	17.4	K-KA	17.2783
8	Calcium (Ca)	47.0	Ca-KA	26.3153
9	Chromium (Cr)	0.166	Cr-KA	0.0453
10	Manganese (Mn)	0.805	Mn-KA	0.3310
11	Iron (Fe)	3.79	Fe-KA	2.3443
12	Zinc (Zn)	0.151	Zn-KA	0.2622
13	Rubidium (Rb)	0.0637	Rb-KA	0.3207
14	Strontium (Sr)	0.0878	Sr-KA	0.5048

NC analysis, using Shimadzu Corporation (Kyoto, Japan)'s Sumigraph NC-220F (performed at Tokyo University of Agriculture and Technology, Fuchu Campus), demonstrated a high concentration of carbon in the tested samples. On average, the collected samples had a carbon content of 44%. Nitrogen was limited to 1.3% on average.

WD-XRF was performed by Japan Testing Laboratories. Around 6 g of the pulverized fermented leaves was sent as a sample for the analysis. For this analysis, Rigaku (Tokyo, Japan)'s ZSX Primus II was used.

Elements ranging from fluorine (F), with an atomic number of 9, to uranium (U), with an atomic number of 92, were analyzed in the sample. The results are presented in mass percentage of the total elements available in the sample ranging from fluorine (F) to uranium (U). The results showed that the material was rich in calcium (Ca), silicon (Si), and potassium (K). Calcium (Ca), comprising around 47% of the total material, represented the highest amount.

2.2. Sea Water

Natural sea water from the Izu Islands was obtained from a local company. Water was extracted from the top 10 m of the sea. The 10 m depth was chosen because it is the most appropriate depth for phytoplankton to live.

2.3. Phytoplankton Alga Culture

Chlorella vulgaris—a kind of phytoplankton—was chosen for the task. Chlorella vulgaris is a green-colored eukaryotic microalga that has a high protein content and is widely used as medicine in a number of countries such as China, Japan and the United States [22].

The alga culture was ordered from a local Japanese shop. The product was received in a frozen condition and used immediately to avoid perishing.

2.4. Concrete Specimen

Three types of concrete were used in this study: conventional concrete (CC), bokashi fermented leaves concrete with 2% of bokashi fermented leaves (BFLC-2) by weight of cement replacing sand by its volume, and bokashi fermented leaves concrete with 20% of bokashi fermented leaves (BFLC-20) by weight of cement replacing sand by its volume.

2.4.1. Concrete Mix Design

Table 4 shows the mix proportions of concrete types used in the study.

Table 4. Concrete mix design.

Concrete Type	Unit (Kg/m^3)				
	Water	**Cement**	**Sand**	**Coarse Aggregate**	**Bokashi Fermented Leaves (BFL)**
CC	180	360	556	1133.5	–
BFLC-2	180	360	529.41	1133.5	7.2
BFLC-20	180	360	290.2	1133.5	72

Since the Bulk SSD Specific Gravity of bokashi fermented leaves (BFL) is very low and directly affects the hydration of cement, it was decided to measure the BFL as the percentage by weight of cement so that hydration could slow down to a limited level. The volume of the BFL then replaced sand.

2.4.2. Specimen Design

We prepared 10 × 10 cm square molds using plywood, and then we applied silicon to make it waterproof. These molds were designed to be prototypes of real-life artificial reefs, so openings were provided.

The final concrete specimens had a similar design, though the surface of BFLC-20 was rough compared with conventional concrete or BFLC-2, which is considered to be a good quality that allows biota to grow on the surface [11].

2.5. pH of Concrete Surface

The pH of surface of the concrete specimens was checked since the pH of the surface plays a huge role in algal growth [3]. Higher pH values are toxic for algae species. The surface pH was simply checked by spraying distilled water on the surface of the concrete and using pH paper to measure the pH. The surface pH values of all the concrete types were checked after 5 months of immersion in seawater.

2.6. Algal Growth

Algal growth was observed on a small scale in the experimental lab since the material development was in its initial stages. Since it was difficult to observe different kinds of algae growth on the specimen due to the limited time, only Chlorella vulgaris was chosen for this study. Chlorella vulgaris is a popular phytoplankton species that is especially used to produce medicines.

2.6.1. Experimental Setup

Specimens were immersed in 120 L of real sea water from the Izu Islands. The sea water was collected from the top 10 m layer of sea. The water was then poured in 120 cm × 90 cm fiber pools, and the concrete specimens were immersed (Figure 2). Three pools with three specimens of CC, BFLC-2, and BFLC-20 were placed inside a greenhouse with favorable temperature and nutritional values of water. In order to provide the necessary carbon dioxide and favorable movement in water for algal growth, aquarium air pumps were used. A greenhouse heater was also installed to maintain a temperature of around 25 °C inside the greenhouse for the algae to thrive.



Figure 2. Immersed samples for algal growth observation. CC (lower right corner), BFLC-2 (upper left corner) and BFLC-20 (upper right corner).

2.6.2. Fertilizer and Nutrient Control

Algae, similar to plants, use photosynthesis to grow and require nitrogen-, phosphorus- and potassium-like substances to thrive in any environment [23]. In order to provide proper nutrition and to maintain the same nutritional values in all three pools, Plant Pack Enhancer NPK from Seachem (Madison, GA, USA) and JBL (Neuhofen, Germany)'s Pro Aquatest was used. Plant Pack Enhancer NPK provides nitrogen, phosphorous, and potassium in highly concentrated forms. JBL's Pro Aquatest is specifically designed for aquariums and comes with a range of chemicals that can predict the amount of nitrogen, phosphorus, and a range of other minerals and compounds inside water.

2.6.3. Image Analysis

Image analysis was performed in order to find out what percentage of the area of the concrete specimens was covered with algae. The image analysis was performed using ImageJ Analysis Software developed by NIH, USA (Bethesda, MD, USA). The pictures of concrete specimens were taken at certain intervals—specifically 15, 20 and 35 days after the addition of the Chlorella vulgaris culture to the water.

The pictures were first converted to an 8-bit color system from RGB so that it would be easier for the software to differentiate based on the intensity of blacks on the surface of concrete specimens. The images were then analyzed for algal coverage using the threshold values.

3. Results and Discussion

3.1. pH of Concrete

From Table 5, it can be seen that BFLC-20 had the lowest pH of 7.5. This could have been because of the rougher and porous surface of the concrete type, which could be observed with the naked eye. The porous surface caused the leaching out of calcium hydroxide (CaOH), therefore reducing the pH of the concrete.

Table 5. pH of the surface of concrete after 5 months of immersion.

Concrete Type	Average pH after 5 Months of Immersion in Water
CC	10
BFLC-2	10
BFLC-20	7.5

As already discussed, higher pH values of concrete surfaces affect algal growth. For this sole reason, researchers and scientists have made different types of concrete that tend to have lower surface pH values using materials such as granulated blast furnace slag and steel slag [10], sulfoaluminate cement [24], and different kinds of pozzolana [11]. The resulting concrete types have shown higher algal growth compared with conventional concrete.

3.2. Algal Growth

The alga culture was added after the concrete specimens were immersed in sea water. Images were taken at 15, 20 and 35 days after the addition of algae in sea water.

The results (see Figures 3 and 4) showed that BFLC-20 had the highest algae cover of around 80%, while CC had the lowest cover of around 2% by the end of the 35 days. BFLC-2 performed a little better than the CC, with around 6% of cover by the end of the 35 days; however, this was not on par with BFLC-20.

We observed that higher the amount of BFL in concrete, the higher the algal coverage. This was because of two reasons, i.e., the pH of the concrete surface and the packed nutrients inside the BFL.



Figure 3. Algal growth on CC, BFLC-2, and BFLC-20 after 35 days.

Research suggests that Chlorella vulgaris tends to grow better in environments where the pH ranges from 7.5 to 8.0 [25].

Similarly, a number of studies have proven that carbohydrates tend to improve algal growth. Additional organic carbon, such as in the form of glucose, glycerol, and fructose, tends to improve the growth of Chlorella vulgaris and/or their lipid production [26–28].

Recent research has tended to show higher algal growth when incorporating nutrients inside concrete [12]. The leaching out and adsorption of nutrients has also been reported in artificial reefs [13,29].

Based on previous research, we assumed that a high amount of carbohydrates—given a high percentage of carbon as per the NC analysis—and amino acids leached out of concrete, after which some amount of them stuck to the surface of the concrete due to ionic attraction chiefly caused by calcium (Ca^{2+}) and hydroxide (OH^{-}) ions [12,30–32].

Other mineral nutrients in BFL (i.e., calcium, silicon and potassium) have led to improvements in algal growth in a number of research studies [33].

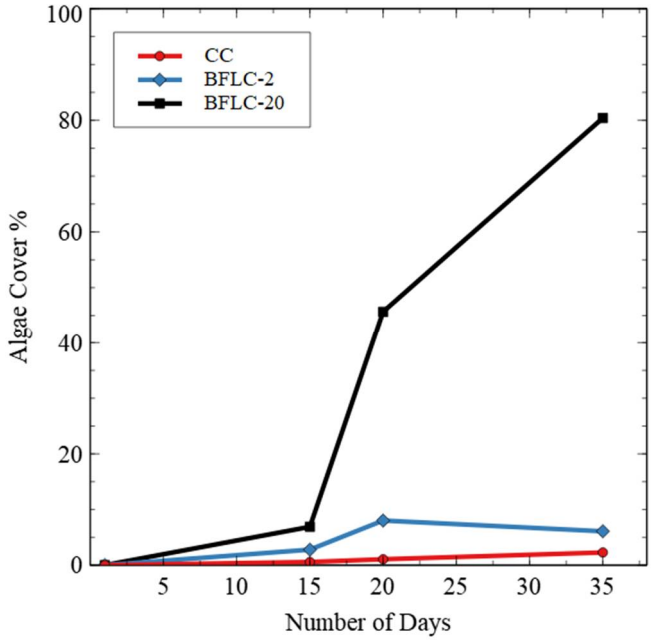


Figure 4. Percentage of algae cover on CC, BFLC-2, and BFLC-20.

4. Recommendations

The scope of the study was limited due to time constraints, and the following research directions are recommended for the future.

1. Various algae species, other than Chlorella vulgaris, should be checked for improvement in growth.
2. Nutrients from other organic sources should be studied.
3. The scope of the study should be expanded, and concrete specimens should be checked for algal growth in the real sea environment.

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