Growth and survival of *Acropora* transplanted on FABA-containing and conventional concrete substrates

Novriadi, Yanti Ariyanti, Syaadatun Nadiah, Nurul Adhha, Muhammad Asril

Faculty of Science, Sumatera Institute of Technology, Lampung Selatan, Lampung, Indonesia. Corresponding author: S. Nadiah, syaadatun.nadiah@fa.itera.ac.id

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Abstract. This study evaluated the suitability of fly ash and bottom ash (FABA) from PT Bukit Asam as a partial cementitious component for coral transplantation substrates. FABA composition was characterized using X-ray fluorescence, and the genus-level identity of transplanted coral fragments was verified using mitochondrial COI and 12S rRNA markers followed by BLASTN analysis. A comparative transplantation experiment was conducted using 60 *Acropora* coral–substrate units, comprising 30 FABA-containing substrates and 30 conventional concrete substrates, with coral growth and survival monitored for six months. FABA was dominated by SiO_2, Al_2O_3, CaO, and Fe_2O_3. Molecular analysis supported assignment of the experimental fragments to the genus *Acropora* but did not provide sufficient resolution for definitive species identification. Initial fragment lengths were comparable between treatments (10.00±0.47 cm for FABA and 9.92±0.49 cm for concrete; p=0.558). Mean absolute length increase after six months was 5.28±0.26 cm on FABA and 5.34 ± 0.26 cm on concrete, with no significant difference between treatments (p=0.367). Growth trajectories also did not differ significantly between treatments (treatment × time, p=0.128). The endpoint survival was 90.0% (27/30) on FABA and 83.3% (25/30) on concrete, with no significant difference between treatments (p=0.706). These results indicate that the tested FABA-containing substrate supported short-term *Acropora* transplantation performance broadly comparable to conventional concrete under the tested conditions. Further assessment of long-term substrate durability and element leaching under prolonged marine exposure is required before broader application.
Keywords: *Acropora*, coral transplantation, FABA, reef restoration.

Introduction. Coral reefs are among the most biologically diverse marine ecosystems and provide important ecosystem services (Hughes et al., 2023). Increasing local and global pressures have contributed to extensive reef degradation, creating a need for active restoration approaches to support coral recovery. Artificial reefs and coral transplantation are among the approaches used in active reef restoration (Ozanam et al., 2025). The characteristics of the substrate are particularly relevant because coral settlement and post-settlement survival can be influenced by the physical and chemical properties of the available surface (Harrington et al., 2004).

The suitability of a substrate for coral transplantation depends not only on the presence of a stable hard surface but also on the characteristics of the substrate itself. Previous studies have shown that substrate composition and surface properties can influence coral settlement and the subsequent performance of transplanted or juvenile corals. Inorganic material composition and localized surface characteristics can affect coral larval settlement, while substrate morphology and composition may influence the growth and propagation of transplanted corals (Levenstein et al., 2022; Matus et al., 2021). In addition, the growth of transplanted *Acropora* fragments can be affected by fragment characteristics and their position or orientation within a transplantation system (Novriadi et al., 2025; Soong & Chen, 2003). These findings indicate that evaluation of an alternative coral transplantation substrate should consider both its material characteristics and the biological response of the coral. A previous study by Novriadi et al. (2025) investigated the potential use of FABA-based substrates for Acropora transplantation at Pahawang Island. However, the present study was based on an independent transplantation experiment

conducted using a separate set of experimental units and coral fragments. No growth or survival data reported in the present study were included in the previous publication. The experiments were also conducted at different field settings: the previous study was performed in the Jelarangan pier area, whereas the present study was conducted at Gosong Bekri, a patch reef environment with different local habitat and environmental conditions. In addition, the present study incorporated material characterization and molecular verification of the experimental coral fragments.

Conventional concrete has been widely used as a material for artificial reef structures because it provides a rigid substrate for coral transplantation and reef development. Experimental transplantation studies have also demonstrated that differences in concrete composition can be associated with differences in coral growth, although not all alternative compositions produce significantly different responses from conventional concrete. For example, *Acropora muricata* fragments transplanted onto conventional concrete and modified concrete substrates showed comparable fragment-lengthening and colony-diameter increments for some substrate formulations (Muzaki et al., 2019) This has encouraged the investigation of alternative materials derived from industrial by-products for artificial reef applications. Fly ash and bottom ash (FABA)-based kerbstone substrates have previously been tested for *Acropora* transplantation, with reported growth and survivorship over several months of field observation (Khasanah et al., 2020).

FABA are coal-combustion residues whose chemical composition can vary substantially depending on the coal source and combustion conditions. Their relatively high contents of silica, alumina, iron oxides, and calcium-bearing phases have supported their use in cementitious and construction applications. However, the presence of trace elements in coal combustion ash also requires consideration when the material is intended for applications involving prolonged contact with water. The environmental behavior of these elements cannot be inferred solely from their total concentrations because their mobility depends on factors such as ash composition, mineral association, and leaching conditions (Izquierdo & Querol, 2012). Therefore, chemical characterization and assessment of elemental mobility are important preliminary steps when evaluating FABA-derived materials for use in marine applications.

A further consideration is the biological consistency of the coral used in substrate experiments. *Acropora* is an important coral genus for reef restoration because of its relatively rapid growth and contribution to reef framework development (Roff, 2020). Molecular identification can therefore be used to verify the taxonomic identity of coral fragments used as experimental units and reduce uncertainty associated with morphological identification, particularly within morphologically similar *Acropora* taxa (Ramírez-Portilla et al., 2022). In the present study, mitochondrial COI and 12S rRNA markers were used to confirm the genus-level identity of the experimental coral fragments (Shinzato et al., 2021).

The critical question, however, is whether partial substitution of cement with FABA alters coral performance. A suitable FABA-based substrate should not reduce coral survival or growth relative to conventional concrete. Therefore, the present study integrates material characterization, molecular identification of the coral test organism, and a comparative transplantation experiment. Specifically, this study aimed to (1) characterize the chemical composition of fly ash (FA) and bottom ash (BA) obtained from PT Bukit Asam Unit Pelabuhan Tarahan; (2) verify the genus-level identity of the *Acropora* fragments used in the experiment; and (3) compare the growth and survival of *Acropora* transplanted onto conventional concrete and FABA-substituted concrete. The results are intended to determine whether FABA can be considered a potential partial substitute for cement in coral transplantation substrates, while identifying the need for further testing before broader field application.

Material and Method

Study site and duration. This study was conducted at Jelarangan Hamlet, Pahawang Island, Pesawaran District, Lampung Province, Indonesia (5°33'42"S, 105°14'27"E). The locations were mapped using WGS 84 / UTM Zone 48S and are shown in Figure 1. The

transplantation experiment was conducted in Jelarangan waters at a depth of 3–5 m, while reef and reef-fish surveys were conducted at the designated survey site. The study was conducted from September 2024 to March 2025 as a collaborative project between PT Bukit Asam Tbk, Unit Pelabuhan Tarahan, and the Institut Teknologi Sumatera (ITERA).

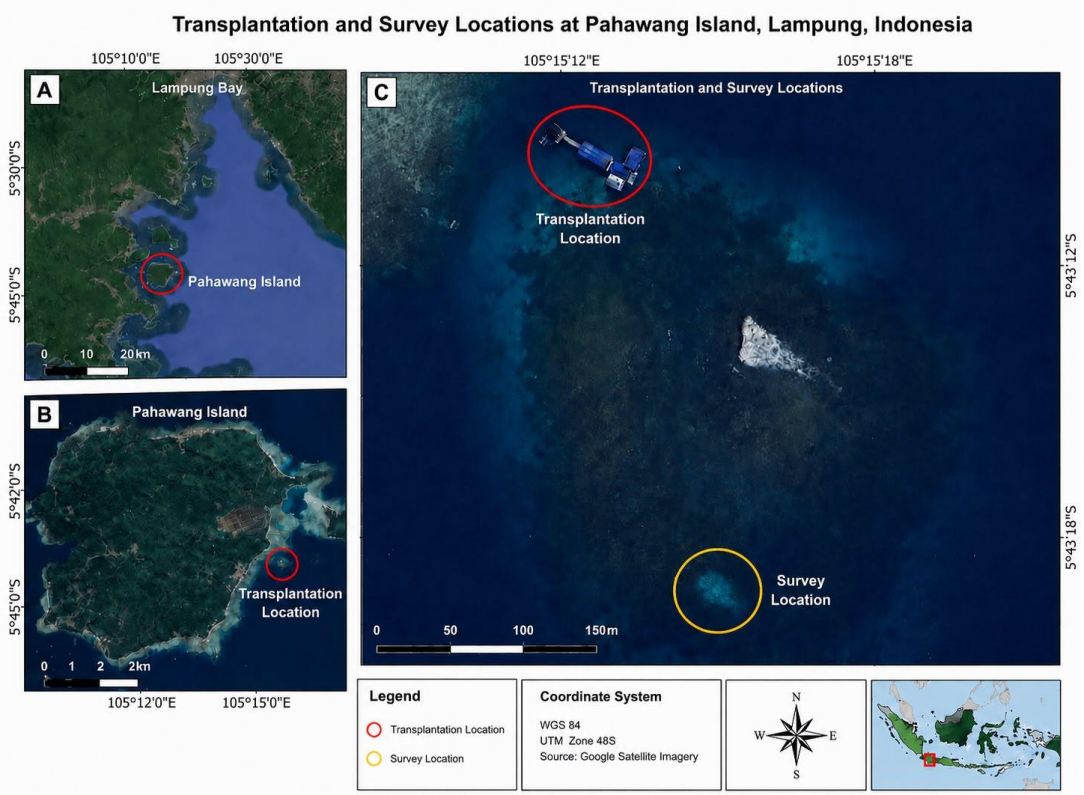


Figure 1. Study area and locations of coral transplantation and reef surveys in Pahawang Island, Lampung, Indonesia. (A) Location of Pahawang Island within Lampung Bay; (B) location of the transplantation site on Pahawang Island; and (C) detailed spatial distribution of the transplantation site and reef survey site.

Characterization of fly ash and bottom ash. The FA and BA used in this study were collected from the coal combustion residue of PT Bukit Asam Unit Pelabuhan Tarahan power plant. Samples were air-dried, homogenized, and analyzed for their chemical composition using X-ray Fluorescence (XRF) spectroscopy at the BRIN Mineral Laboratory, Lampung. The concentrations of oxides including SiO_2, Al_2O_3, CaO, Fe_2O_3, and trace metals such as Pb, Cd, Hg, and As were determined. These analyses aimed to evaluate the material's suitability as a partial cement substitute for coral transplantation substrate fabrication.

Coral sampling and DNA identification. Coral fragments of *Acropora* sp. were collected from three sites around Pahawang Island at depths of 3–7 m. Three colonies from each site were sampled, and fragments of approximately 3 cm were preserved for molecular identification. DNA extraction was performed using a DNeasy Blood & Tissue Kit (Qiagen), followed by amplification of mitochondrial COI and 12S rRNA gene regions via Polymerase Chain Reaction (PCR) using scleractinian-specific primers that were developed for environmental DNA (eDNA) metabarcoding. (Scle_CO1_Fw (5'-ATT GTN TGR GCN CAY CAT ATG TTT A-3') and Scle_CO1_Rv (5'-CCC ATA GAR AGN ACA TAR TGA AA-3') and the 12S rRNA gene with Scle_12S_Fw (5-CCAGCMGACGCGGTRANACTTA-3) and Scle_12S_Rv (5-AAWTTGACGACGGCCATGC-3) (Shinzato et al., 2021) . Amplified products were visualized using agarose gel electrophoresis and sequenced. Sequence homology was analyzed using NCBI BLAST to confirm genus identity.

Fabrication of FABA-based substrate and transplant experiment. A coral transplantation experiment was conducted to evaluate the feasibility of FABA as a substrate material. FABA-based substrates were prepared by incorporating FABA into a cement–sand mixture at a 1:1 ratio, resulting in a final composition of 20% FABA and 80% cement–sand. The mixture was molded into circular discs (10 cm diameter × 2.5 cm thickness), with a PVC pipe (2 cm diameter × 5 cm length) vertically embedded at the center as a coral fragment holder. The substrates were cured for 20 days before deployment.The conventional control substrates were prepared from SikaCeram®-255 Big Slab, sand, and water at proportions of 1.0 kg, 3.0 kg, and 0.50 kg (approximately 500 mL), respectively. The mixture was molded into discs with the same dimensions and PVC holder configuration as the FABA-based substrates and cured for 20 days. Coral fragments of *Acropora* (approximately 10 cm in length) were inserted into the PVC holder and secured using SikaCeram®-255 Big Slab. The same adhesive was used for both substrate treatments. measurements of coral length and survival. A total of 60 coral–substrate units were prepared, comprising 30 FABA-based and 30 conventional concrete substrates. Each unit was assigned a unique identification number and randomized as a single sequence before deployment. The first 30 units were assigned to Frame 1 and the remaining 30 to Frame 2. The two frames were deployed at approximately 3–4 m depth. Each coral–substrate unit was treated as an individual experimental unit for repeated

Data analysis. Coral fragment length was summarized as mean ± standard deviation (SD) for each substrate treatment and observation period. Initial fragment length was compared between FABA-based and conventional concrete substrates using Welch's t-test. Longitudinal changes in fragment length were evaluated using a repeated-measures mixed-effects model with substrate treatment, observation time, and their interaction as fixed effects, with individual coral fragments considered as repeated observational units. The treatment × time interaction was used to assess whether coral growth trajectories differed between substrate types. Survival at the end of the six-month monitoring period was calculated as the proportion of fragments remaining alive. Differences in endpoint survival between treatments were evaluated using Fisher's exact test. Mortality and was recorded separately. Statistical significance was set at $p=0.05$.

Ethical and environmental considerations. No endangered coral species were harmed during the process, and transplantation was performed with community participation from Pahawang residents to ensure sustainability and knowledge transfer.

Results

Chemical composition of fly ash and bottom ash. The X-Ray Fluorescence (XRF) analysis of FA and BA from PT Bukit Asam Unit Pelabuhan Tarahan revealed major cementitious oxides: SiO_2, Al_2O_3, CaO, and Fe_2O_3. The SiO_2 concentration reached 53.79% in FA and 52.46% in BA, confirming strong pozzolanic characteristics, while CaO was 9.50% in FA and 15.08% in BA, contributing to binding properties. The chemical composition of FA and BA, determined by XRF analysis, is summarized in Table 1.

Table 1

Chemical composition of fly ash (FA) and bottom ash (BA)

Oxide component	*FA (%)*	*BA (%)*
SiO_2	53.79	52.46
Al_2O_3	23.95	9.85
CaO	9.50	15.08
Fe_2O_3	12.73	22.85

The XRF analysis indicates that both FA and BA are dominated by silica-based compounds, reflecting their pozzolanic characteristics and potential suitability as cementitious materials. Variations in the relative proportions of alumina, calcium oxide, and iron oxide

between FA and BA suggest differences in their binding and structural properties. The relative proportion of major oxide components in FA and BA is illustrated in Figure 2.

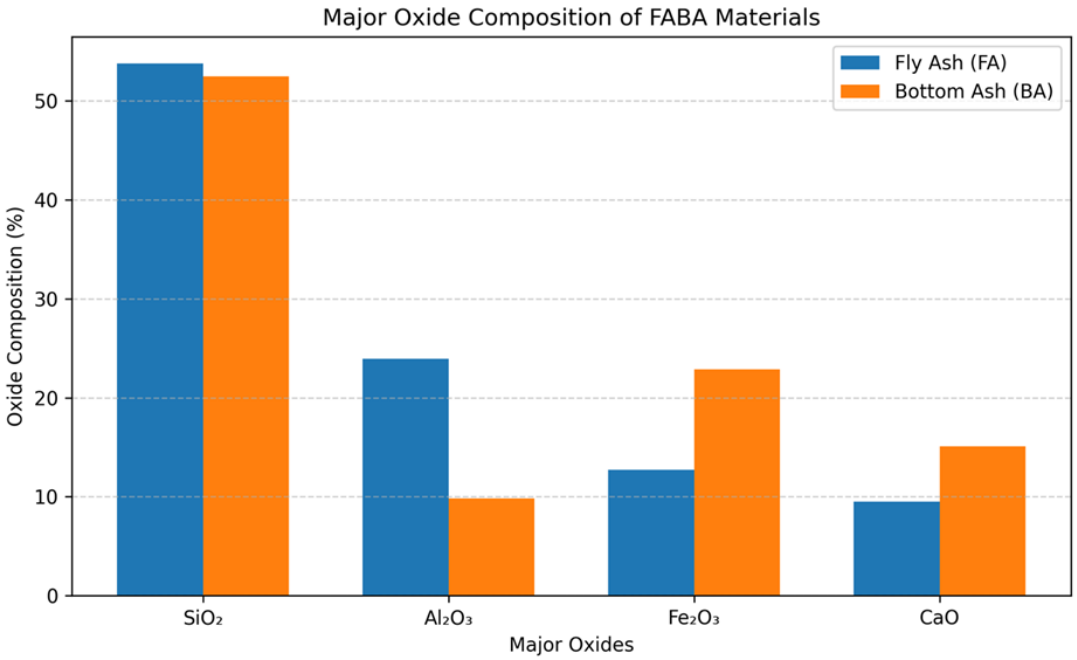


Figure 2. Major oxide composition in FABA materials (A bar chart showing that both FA and BA are dominated by SiO_2, confirming their potential as pozzolanic materials).

Major oxide composition analysis of FA and BA revealed that both materials are predominantly composed of silica (SiO_2), accounting for 53.79% and 52.46%, respectively. This dominance of SiO_2 is a key characteristic of pozzolanic materials, which are known for their ability to react with calcium hydroxide in the presence of water to form cementitious compounds. In addition to SiO_2, significant amounts of Al_2O_3 and Fe_2O_3 were detected, further supporting the classification of FABA as a silico-aluminous material. The presence of CaO, although lower than that found in Portland cement, may contribute to early-stage bonding and substrate stability. These compositional characteristics indicate that FABA possesses suitable pozzolanic properties, supporting its potential application as an environmentally friendly alternative material in coral transplantation substrates.

Trace elements such as As, Cd, Hg, Pb, and Cu were detected at low total concentrations in the FABA samples based on XRF analysis. However, these values represent total elemental concentrations in the solid matrix and cannot be directly compared with marine water quality standards, which are based on dissolved concentrations in water. Therefore, the XRF results alone do not establish the leaching behavior, bioavailability, or environmental risk of these elements. (Menteri Negara Lingkungan Hidup, 2004). Further leaching tests under seawater or simulated marine conditions are required to evaluate the environmental compatibility of FABA for marine applications.

Coral DNA identification. Coral fragments collected from Pahawang waters were successfully amplified using COI and 12S rRNA mitochondrial markers. Electrophoresis showed clear DNA bands of 298-299 bp for COI and 458-459 bp for 12S rRNA, confirming successful amplification. Preliminary taxonomic validation was conducted using BLASTN. For each gene (COI and 12S rRNA), sequences from 28 *Acropora* species were retrieved from the NCBI database. The top matches showed high sequence homology, averaging 96% for COI and 93% for 12S rRNA.

Coral transplantation. A total of 60 *Acropora* fragments were monitored for six months, comprising 30 fragments transplanted onto FABA-based substrates and 30 onto conventional concrete substrates. Initial fragment length did not differ significantly

between treatments, with mean initial lengths of 10.00±0.47 cm for FABA and 9.92±0.49 cm for concrete (Welch's t-test, p=0.558). After six months, mean absolute length increase was 5.28±0.26 cm for FABA and 5.34±0.26 cm for concrete, with no significant difference between treatments (p=0.367). The longitudinal analysis showed no significant treatment × time interaction (p=0.128), indicating that the temporal growth trajectories of coral fragments did not differ significantly between FABA-based and conventional concrete substrates. At the end of the six-month monitoring period as shown in Figure 3, 27 of 30 fragments on FABA-based substrates remained alive (90.0%), compared with 25 of 30 fragments on conventional concrete substrates (83.3%). Fisher's exact test showed no significant difference in endpoint survival between treatments (p=0.706).

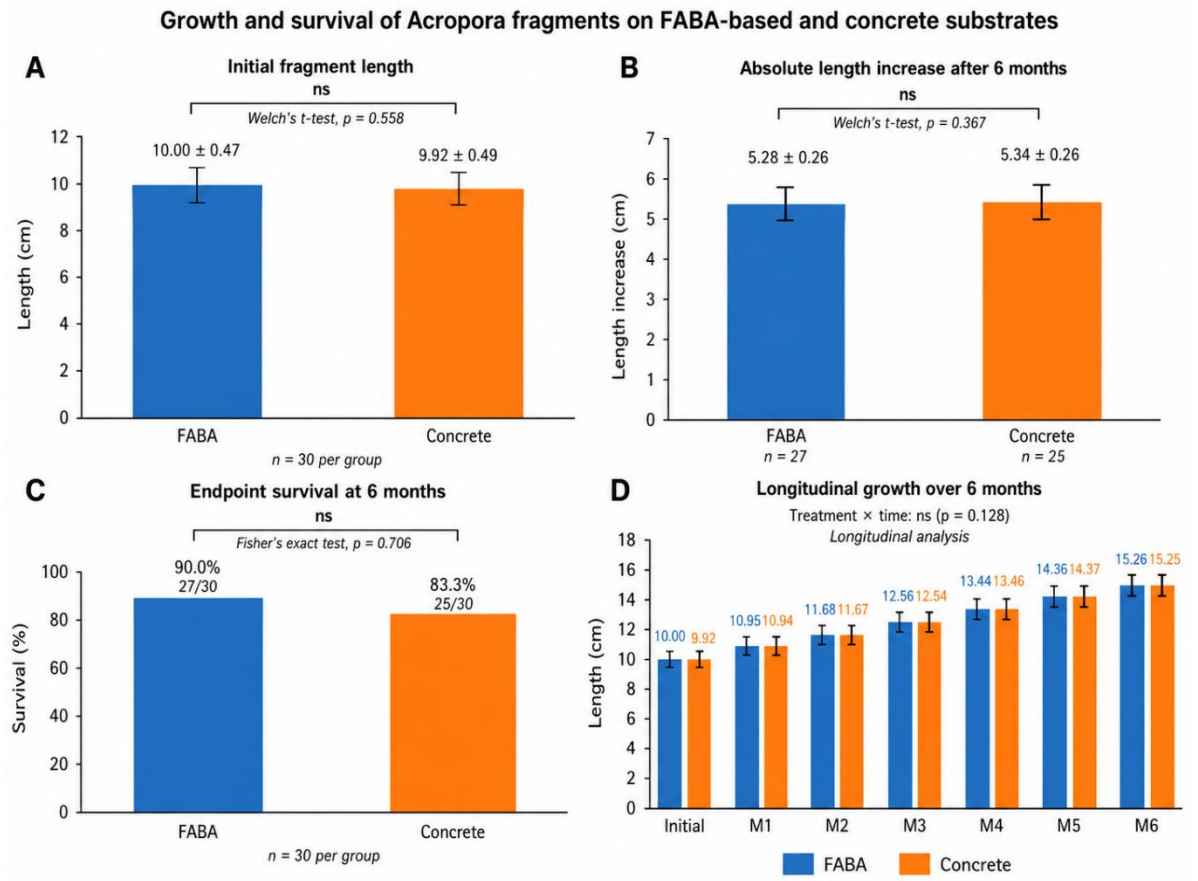


Figure 3. Growth and survival of *Acropora* fragments on FABA-based and conventional concrete substrates during six months of transplantation.

The details are as follows: (A) Initial fragment length was comparable between the FABA and concrete groups (10.00±0.47 vs. 9.92±0.49 cm, respectively; Welch's *t*-test, *p*=0.558; *n*=30 per group). (B) Absolute length increase after six months did not differ significantly between FABA (5.28±0.26 cm; *n*=27) and concrete substrates (5.34±0.26 cm; *n*=25; Welch's *t*-test, *p*=0.367). (C) Endpoint survival was 90.0% (27/30) in the FABA group and 83.3% (25/30) in the concrete group, with no significant difference between treatments (Fisher's exact test, *p*=0.706). (D) Longitudinal changes in fragment length from baseline to month 6 showed progressive growth in both groups, with no significant treatment × time interaction (*p*=0.128), indicating similar temporal growth trajectories between substrates. Bars represent mean ± standard deviation (SD), except for survival data, which are presented as percentages. ns, not significant (*p*>0.05).

Representative images of *Acropora* fragments transplanted onto FABA-based and conventional concrete substrates during the six-month monitoring period are presented in Figures 4 and 5.

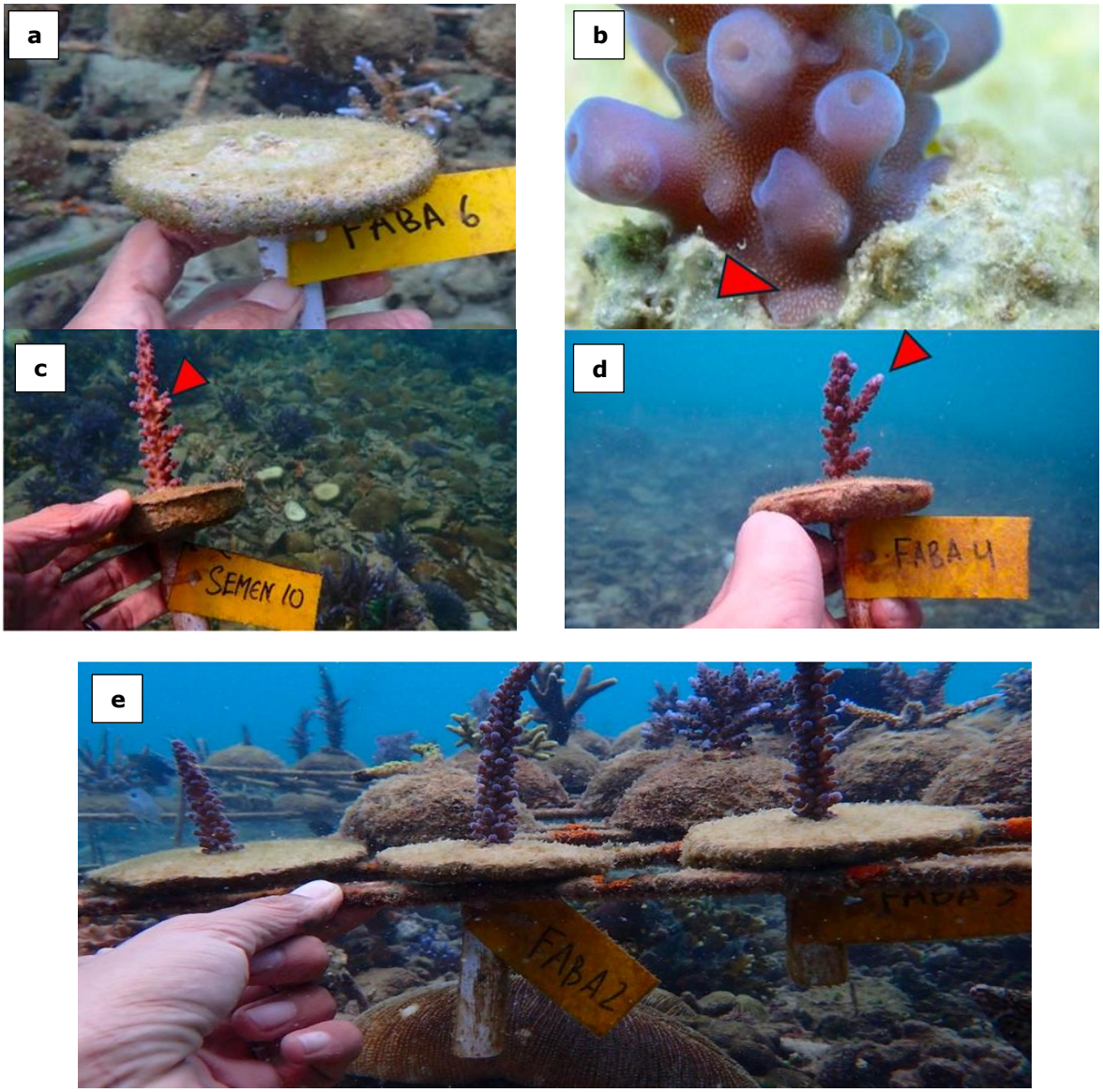


Figure 4. Growth progression of *Acropora* on FABA substrate: (a) Detached fragment, (b) Basal tissue growth, (c) New branch of Semen, (d) New branch of FABA (e) Fully attached colony after three months.



Figure 5. Deployment of FABA-coated reef domes and metal frames in Jelarangan waters.

Discussion. The chemical composition of coal combustion ash is an important consideration when assessing its potential incorporation into cementitious materials, but the presence of major oxide components alone is insufficient to establish pozzolanic performance. The cementitious contribution of fly ash depends not only on its bulk SiO_2, Al_2O_3, Fe_2O_3, and CaO contents, but also on the mineralogical form, proportion of amorphous phases, particle size, and accessible reactive phases (Li et al., 2021). Differences in these characteristics can result in substantial variation in pozzolanic reactivity among ashes with apparently similar chemical compositions. Experimental studies have demonstrated that physical processing, such as classification and milling, can substantially alter the measured reactivity of fly ash, further indicating that chemical composition should be interpreted together with mineralogical and physical properties (Snellings et al., 2021; Tkaczewska, 2021).

In the context of the present study, the FABA composition therefore provides evidence that the material has characteristics relevant to cementitious applications, but it does not by itself demonstrate strong pozzolanic reactivity. This distinction is particularly important because the substrate used in the transplantation experiment was a FABA-modified cementitious material rather than FABA used as a standalone binder. The observed performance of the transplantation substrate should consequently be interpreted as the performance of the formulated composite, rather than attributed directly to the pozzolanic activity of FABA. A more definitive assessment of its cementitious contribution would require complementary analyses such as X-ray diffraction to determine mineralogical and amorphous phases, together with direct pozzolanic-reactivity and mechanical-performance tests.

The environmental interpretation also requires caution. The presence of trace elements in coal combustion ash does not necessarily indicate environmental toxicity, because their environmental significance depends on their chemical speciation, mineral association, and mobility under the conditions of exposure (Gopinathan et al., 2022). Total concentrations alone are not reliable indicators of environmental risk because leaching behavior is strongly influenced by ash characteristics and solution chemistry (Izquierdo & Querol, 2012). This consideration is particularly relevant to a marine transplantation application, where prolonged contact with seawater may alter the mobility of some elements. Thus, the present chemical characterization should be regarded as an initial screening step rather than definitive evidence of long-term environmental safety. This interpretation provides a more conservative assessment of the FABA material (Zhang et al., 2022). In particular, the present XRF data demonstrate its chemical composition but do not by themselves demonstrate strong pozzolanic reactivity, mechanical suitability, or long-term environmental safety. These distinctions are important when considering FABA as a partial cementitious component for coral transplantation substrates (Chen et al., 2024).

Coral DNA molecular identification provided an independent taxonomic basis for defining the coral material used in the transplantation experiment. This is particularly relevant for *Acropora*, in which morphological similarity, phenotypic variation, and evolutionary processes such as hybridization and incomplete lineage sorting can complicate species delimitation (Ramírez-Portilla et al., 2022). The morphological species boundaries in *Acropora* do not always correspond to genetically distinct evolutionary units, emphasizing the difficulty of relying exclusively on morphology for taxonomic assignment (Van Oppen et al., 2001).

The use of mitochondrial COI and 12S rRNA therefore provides useful molecular support for assigning the experimental fragments to *Acropora* at the genus level. Shinzato et al. (2021) specifically developed mitochondrial 12S and COI markers for scleractinian corals and demonstrated their utility for genus-level identification and metabarcoding. However, the same study also showed that mitochondrial sequences are highly conserved among scleractinian corals, limiting their discriminatory power at finer taxonomic levels. This limitation is consistent with the present study, in which the molecular data supported assignment to *Acropora* but did not provide sufficient resolution for definitive species identification. Recent work further demonstrates that even commonly used DNA barcoding markers can be insufficient for reliable species-level identification within *Acropora*, with

substantial overlap among closely related taxa. Therefore, the molecular results should be interpreted as genus-level taxonomic support for the experimental coral material, rather than as definitive species identification (Quek et al., 2024).

The six-month experiment of coral transplantation on FABA and concrete substrates indicates that the FABA-containing composite substrate supported continued growth and survival of *Acropora* fragments at a level comparable to the conventional concrete substrate. The similar trajectories of fragment elongation between treatments suggest that the incorporation of FABA into the cementitious substrate was not associated with an obvious reduction in coral growth over the six-month observation period. This finding is consistent with previous transplantation studies demonstrating that *Acropora* fragments can grow on artificial substrates, including concrete, iron, bamboo, and FABA-based kerbstone (Khasanah et al., 2020; Mompala et al., 2017) .

The comparable growth response between treatments is particularly relevant because the experimental comparison was made between a conventional concrete substrate and a composite substrate containing FABA. The results therefore provide preliminary evidence that partial incorporation of FABA was not associated with a measurable reduction in the biological performance of the composite substrate during the six-month observation period. However, the dataset does not allow the observed growth response to be attributed specifically to the chemical constituents of FABA. Coral growth after transplantation is influenced by several interacting factors, including fragment condition, attachment and substrate surface characteristics (Lewis et al., 2022). Because these variables were not independently manipulated or quantified in the present experiment, the observed response should therefore be interpreted at the level of the composite substrate treatment rather than as a direct physiological effect of FABA.

The survival pattern also indicates that reduced survival was not restricted to the FABA treatment. This is important because the absence of an obvious reduction in survival on the FABA-containing substrate provides preliminary evidence that the formulation was not associated with substantial short-term reductions in transplant performance. Nevertheless, mortality and detachment represent different outcomes and should not be combined as equivalent events (Nithyanandan et al., 2017). A detached fragment may have been lost from the experimental unit without necessarily experiencing tissue mortality. Therefore, future analyses should distinguish survival and mortality when evaluating substrate performance.

The growth data further indicate that the biological response should be evaluated longitudinally rather than from a single endpoint measurement. Monthly measurements allow the trajectory of fragment elongation to be considered, which is more informative than comparing only initial and final length. Previous *Acropora* transplantation studies have similarly used repeated measurements of fragment length to evaluate growth performance across different artificial substrates (Antou et al., 2019). Importantly, the present results should not be interpreted as evidence that FABA is inherently superior to concrete. The available data support a more limited conclusion: the FABA-containing composite substrate supported sustained *Acropora* growth and survival over six months at a performance comparable to the concrete treatment. This distinction is important because the experiment was designed to evaluate a formulated substrate, not to isolate the effect of individual FABA components. A stronger claim of substrate superiority would require robust statistical comparison of repeated growth trajectories and additional evidence on attachment performance, substrate physicochemical properties, and longer-term survival.

The results are therefore relevant primarily as evidence of short-term biological feasibility. They indicate that FABA can be incorporated into the tested coral-transplantation substrate formulation without an obvious deterioration in coral performance relative to conventional concrete during the six-month observation period. However, longer-term monitoring is required to determine whether the observed performance is maintained and whether prolonged seawater exposure alters substrate integrity or releases potentially mobile elements. Such assessment is important because bulk chemical characterization alone does not determine the long-term mobility or environmental behavior of elements under seawater exposure.

Conclusions. This study demonstrates the short-term feasibility of the tested FABA-containing composite substrate for *Acropora* transplantation. Under the experimental conditions evaluated, *Acropora* growth and survival showed no significant difference between FABA-containing and conventional concrete substrates. These findings support the potential use of FABA as an alternative component in coral transplantation substrates while providing a beneficial application for this industrial by-product. Further long-term assessment of substrate durability, elemental leaching, and environmental compatibility under seawater exposure before broader application in coral restoration.

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Author Contributions. Conceptualization: N; Methodology: N and YA; Formal analysis: YA and MA; Investigation: N and NA; Data curation: N; Writing – original draft: SN and N; Writing – review & editing: N and SN; Supervision: N.

Conflict of Interest. The authors declare that there is no conflict of interest.

Data Availability. The data supporting the findings of this study are available from the corresponding author upon reasonable request.

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Authors:
Novriadi (N), Department of Biology, Faculty of Sciences, Sumatera Institute of Technology, Terusan Ryacudu, 35365, Lampung Selatan, Lampung, Indonesia, e-mail: novriadi@bi.itera.ac.id
https://orcid.org/0009-0001-4861-1973
Yanti Ariyanti (YA), Department of Biology, Faculty of Sciences, Sumatera Institute of Technology, Terusan Ryacudu, 35365, Lampung Selatan, Lampung, Indonesia, e-mail: yanti.atiyanti@bi.itera.ac.id
https://orcid.org/0000-0003-4906-135X
Syaadatun Nadiah (SN), Department of Pharmacy, Faculty of Sciences, Sumatera Institute of Technology, Terusan Ryacudu, 35365, Lampung Selatan, Lampung, Indonesia, e-mail: syaadatun.nadiah@fa.itera.ac.id
https://orcid.org/0000-0001-8410-2132
Nurul Adhha (NA), Department of Biology, Faculty of Sciences, Sumatera Institute of Technology, Terusan Ryacudu, 35365, Lampung Selatan, Lampung, Indonesia, e-mail: nurul.adhha@bi.itera.ac.id
https://orcid.org/0000-0002-4943-6417
Muhammad Asril (MA), Department of Biology, Faculty of Sciences, Sumatera Institute of Technology, Terusan Ryacudu, 35365, Lampung Selatan, Lampung, Indonesia, e-mail: muhammad.asril@bi.itera.ac.id
https://orcid.org/0000-0001-8637-4190
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