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Review

Sponge–Microbial Symbiosis and Marine Extremozymes: Current Issues and Prospects

Praise Tochukwu Nnaji *, H. Ruth Morse, Emmanuel Adukwu and Rachael U. Chidugu-Ogborigbo

Department of Applied Sciences, Frenchay Campus, University of the West of England, Bristol BS16 1QY, UK; ruth.morse@uwe.ac.uk (H.R.M.); emmanuel.adukwu@uwe.ac.uk (E.A.); rachael.chidugu-ogborigbo@uwe.ac.uk (R.U.C.-O.)

* Correspondence: praise.nnaji@uwe.ac.uk

Abstract: Marine microorganisms have great potential for producing extremozymes. They enter useful relationships like many other organisms in the marine habitat. Sponge–microbial symbiosis enables both sponges and microorganisms to mutually benefit each other while performing their activities within the ecosystem. Sponges, because of their nature as marine cosmopolitan benthic epifaunas and filter feeders, serve as a host for many extremophilic marine microorganisms. Potential extremozymes from microbial symbionts are largely dependent on their successful relationship. Extremozymes have found relevance in food processing, bioremediation, detergent, and drug production. Species diversity approach, industrial-scale bioremediation, integrative bioremediation software, government and industrial support are considered. The high cost of sampling, limited research outcomes, low species growth in synthetic media, laborious nature of metagenomics projects, difficulty in the development of synthetic medium, limited number of available experts, and technological knowhow are current challenges. The unique properties of marine extremozymes underpin their application in industry and biotechnological processes. There is therefore an urgent need for the development of cost-effective methods with government and industry support.

Keywords: extremozymes; sponges; symbiosis; marine; biotechnology; bioremediation; food processing; industry

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Citation: Nnaji, P.T.; Morse, H.R.; Adukwu, E.; Chidugu-Ogborigbo, R.U. Sponge–Microbial Symbiosis and Marine Extremozymes: Current Issues and Prospects. *Sustainability* **2022**, *14*, 6984. https://doi.org/10.3390/su14126984

Academic Editor: Helvi Heinonen-Tanski

Received: 31 August 2021
Accepted: 23 May 2022
Published: 7 June 2022

1. Introduction

The ocean, sea, brackish water of coastal estuaries, saltwater ponds, and lakes are marine habitats and support the growth of various marine microbes. Marine bacteria constitute a major fraction of the marine biodiversity. This habitat constitutes about 70% of the earth's surface [1–3]. Marine microorganisms enter various relationships with other organisms to survive in their environment (Figure 1). Sponge–microbial symbiosis is one of these relationships [4]. Sponges, as cosmopolitan organisms, rely on ubiquitous microorganisms in marine habitats for certain metabolic processes [5,6]. Sponges, because of their nature as filter feeders, possess a large number of spores for nutrition and metabolism. Therefore, microorganisms that are resistant to the sponge's digestive system and immunity tend to remain within the sponge, [6]. Numerous species of microbes, such as heterotrophic bacteria, cyanobacteria, green and red algae, cryptophytes, etc., have been identified in symbiosis with marine sponges that dwell in various benthic locations of the marine environment (Table 1). Sponges are important members of the benthic ecosystem; they comprise a large proportion of the biomass of the coral reef community, with approximately 98% abundance in marine environments and are available all year round [6,7].

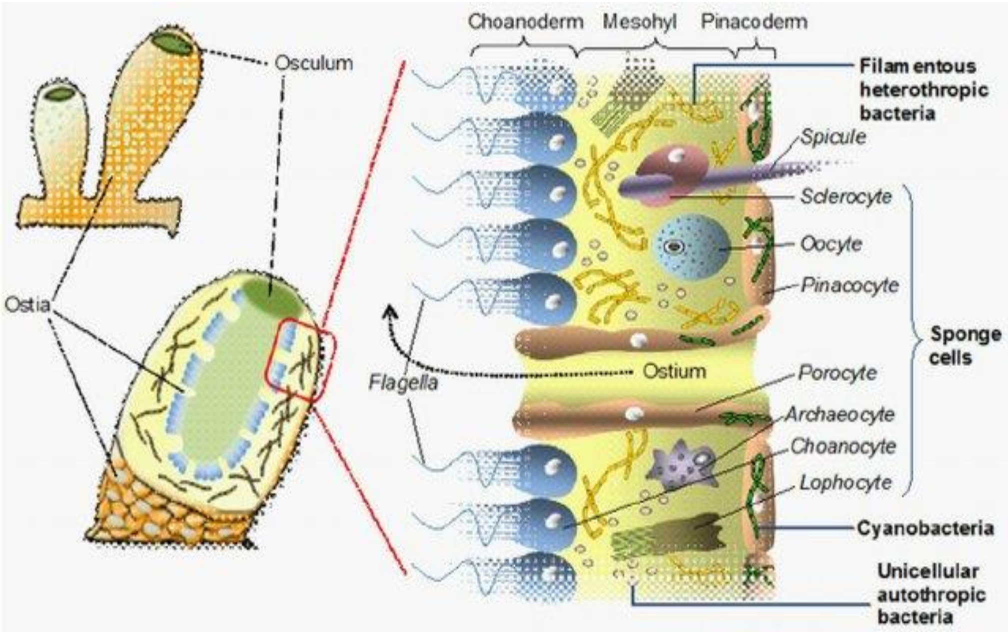

Figure 1. Symbiotic bacterial in sponge tissue. Source: This figure by courtesy of Agustinus R. Uria [4].

Table 1. List of extremophilic microorganisms from the marine habitat and their peculiar extreme characteristics.

Extremophilic Microbes	Marine Habitat	Extreme Characteristics	References
Thermococcus piezophilus CDGS *Pyrococcus*, *Thermotoga*, *Thermococcus*, *Archeoaglobus*, *Methanococcus*, *Pyrodictium*, *Aquifex*	Deep-sea hydrothermal vent Shallow-water and deep-sea hydrothermal vents		[8] [9]
Planococcus halocryophilus Or1	Sea ice core	Halo-psychrophilic	[8]
Halarsenatibacter silvermanii SLAS-1	Soda lake	Halo-alkaliphilic	[8]
Cyanobacteria, *Ceratoporella nicholsoni*, *Rhopaloides odorabile*, *Aplysina aerophoba* *Cytophaga*, and *Desulfovibrio* *Micrococcus species*	Marine sponges Marine sponges		[10] [5]
Salicola, *Halovibrio*, *Halomonas*, *Bacillus*, *Oceanobacillus*, *Thalasobacillus*, *Virgibacillus*, *Gracilibacillus*, *Halobacillus*, *Piscibacillus* and *Salinicoccus*	Howz Soltan Lake	Halophilic	[11]

Microorganisms are known to be reliable sources of enzymes due to their consistency, economic feasibility, high yield, and ease of modification and optimization in comparison to other sources such as plants and animals [12–17]. Enzymes are protein molecules found in cells of living organisms. Over four thousand biochemical reactions, ranging from the digestion of food to the synthesis of DNA, are catalyzed by enzymes [2,12]. Enzymes have a myriad of applications in modern industries; the food, clinical, medical, analytical chemistry, detergent, textile, brewing, and distilling industries apply enzymes in their processes [18]. Despite the high demand for enzymes in various industries, the use of enzymes still faces some peculiar challenges, such as deactivation at extreme temperatures, pressures, salt concentration, pH levels, and high substrate concentration, etc. Regardless of these current challenges, inorganic catalysts are still not a preferred option in biologically related processes for many industries. Instead, there is the search for enzymes with improved qualities of extreme stability called extremozymes. Extremozymes possess exceptional properties beyond their conventional counterparts. They are known to resist deactivation when exposed to extreme conditions such as high temperature, pressure, pH, substrate concentration, salinity, denaturing agents, and organic solvents that denature other enzymes [19] (Table 2).

Table 2. Extremophilic properties and microbial sources of extremozymes.

Extremozymes	Extremophilic Property	Sources	References
Amylase	Thermophilic	*Bacillius*, *Clostridia*, *Fervidobacterium*	[20]
β-Galactosidase	Psychrophilic	*Alteromonas* sp. ML117	[21]
Lipase	Halophilic	*Salicola marasensis* *Marinobacter lipolyticu*	[22,23]
Esterase	Psychrophilic	*Acinetobacter* sp.	[20]
α-Amylase	Thermophilic	*Thermophilic* *Anoxybacillus* sp.	[21]
α-Amylase	Thermophilic	*Bacillus mojavensis* SO-10	[21]
Protease	Halophilic	*Salicola marasensis*	[21]
α-Amylase	Halophilic	*Zunongwangia profunda*	

Sponges in extreme marine environments serve as hosts for symbiotic microorganisms. They possess large numbers of spores on their body since all sponges are filter feeders. Both the sponges and the symbiotic microorganisms produce enzymes for metabolism. Therefore, their ability to successfully thrive in extreme habitats such as the oceanic basement and other benthic regions of the marine ecosystem, indicating that their enzymes have great likelihood of possessing extreme properties. This review will discuss the benefits, prospects, and challenges associated with sponge–microbial symbiosis, and the use of extremozymes in industrial and biotechnological applications.

2. Microbial Biodiversity and Marine Sponge Symbiosis

The marine microbial biodiversity includes bacteria, fungi, viruses, and protists with varying morphological, physiological, and ecological characteristics [24]. Often, these microbes enter into one or more forms of relationships to enable them to survive and reproduce. These relationships could be between two microorganisms or between micro- and macro-organisms such as sponges. The marine habitat has approximately eight thousand species of sponges compared to approximately one hundred and fifty species that are present in freshwaters. *Calcarea, Demospongiae*, and *Hexactinellida* are the three classes of sponges that demonstrate bathymetric differences in abundance within the marine habitat [25]. Marine microbes are distributed at a rate of 10^5 cells in every one milliliter (mL). They inhabit the surface of water and are found in the very deepest areas of the oceans or the deep ocean floor. *Pseudomonas*, *Vibrio*, *Achromobacter*, *Flavobacterium*, and *Micrococcus* species comprise the majority of marine microbial biomass [2,24]. Many marine microorganisms and sponges (especially glass sponges) are found in the dark part of the ocean which does not receive sunlight. Sponges also are found in the benthic regions (Figure 2), which span from the epipelagic coastal region to the hadalpelagic zone, characterized by extreme environmental conditions [25,26].

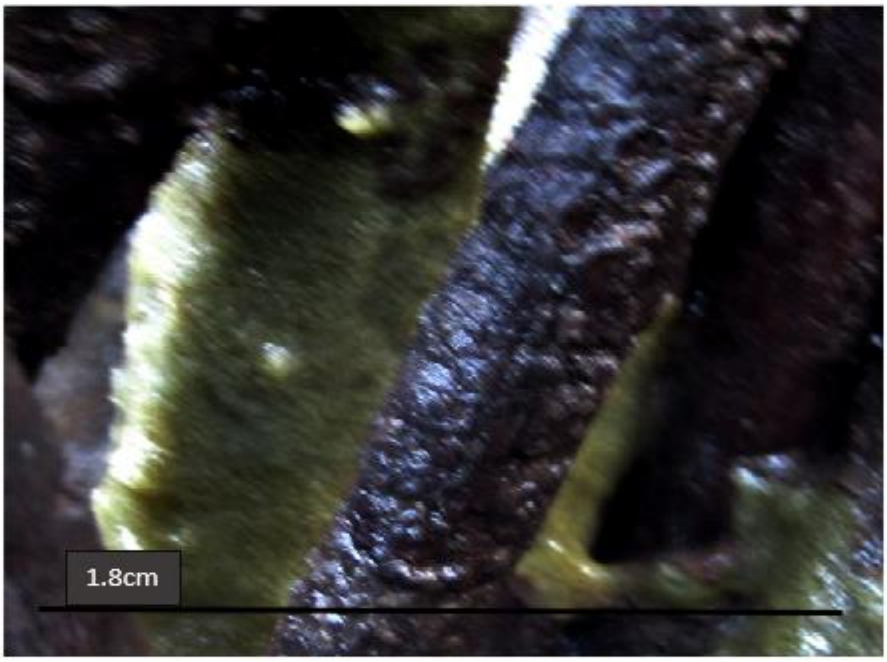

Figure 2. Sponge growth in marine benthic region. Source: [27].

Coral reefs are a complex undersea community bustling with life. They are a sponge–microbial hotspot that leads to the production of mucus containing polysaccharides and proteins. Bacteria, algae, viruses, and single-celled protists inhabit the coral reef [2,7,25,28]. Researchers over the years have discovered that the successful relationship and lifestyle of these organisms in the coral reef influences some of the processes in the Earth's environment. They are reported to be the key ecosystem engineers, providing a conducive habitat and nutrient cycle for the ecosystem. This unique ability of sponges is only guaranteed because of their symbiotic relationship with diverse microbial communities in sponge halobionts [29]. The sponge–microbe symbiosis is such that microbes are found both intracellularly and extracellularly. Extra-symbionts are found in the outer layers while the intra-symbionts are found in the host cell or nuclei [6] While the sponges serve as host for microbes, the sponge microbiome ensures that sponge health and nutrition is intact [29]. Against this backdrop, sea sponges in synergism with their microbial symbionts are proposed to be very useful on an industrial scale bioremediation process especially in polluted marine environments.

3. Benefits of Enzyme Production from Marine Extremophiles

Microorganisms are the most economically feasible and consistent approach to production of enzymes in industrial scale. Under favorable synthetic conditions, they provide high yield irrespective of seasonal differences. In other words, microorganisms have been tried, tested, and trusted as the option for industrial enzyme production [12,13,16,17,30,31]. Since microorganisms are ubiquitous, it would have been much easier to solely depend on microbes from our immediate environment. The natural extent to which an enzyme is developed is largely dependent on the environment of the organism. Therefore, enzymes produced by mesophilic organisms mostly do not possess hyperthermostability when exposed to very high temperature. The deactivation and inactivity of enzymes due to exposure to extreme conditions, which could be sometimes difficult to avoid, is a serious limitation to industries and biotechnological processes. These limitations have resulted in the search for novel enzymes with good tolerance to heat, pH, and pressure [2,18,32,33].

The marine biota is characterized by continuous growth of mean taxa due to the orderly accumulation of evolutionary traits of physiologically buffered and adaptable features with powerful mechanisms of adaptation. These accumulated traits that are acquired from the environment (more especially the bathypelagic, abyssolpelagic, and hadalpelagic zones) are transferred to their offspring, resulting in the breeding of the fittest creatures that can survive the extremities of their environments. Hence, marine microbes and the enzymes they secrete have well-developed adaptive mechanisms for surviving extreme environments [2] that will meet biotechnological and industrial demands. Physiological properties, such as high-pressure tolerance, hyperthermostability, salt and pH tolerance, adaptivity to extreme conditions, and stereochemical properties possessed by enzymes from such extremophiles are desirable in the food, cosmetics, medical, environmental, analytical chemistry, detergent, textile, brewing, and biotechnological industries [34]. Research has shown that enzymes from marine microorganisms have the potential to solve some of the enzyme-related challenges facing bioremediation, food processing, and biotechnological industries (See Table 2 for extremozymes, properties, and respective microbial sources).

4. Marine Extremozymes and Biotechnological Applications

In the agriculture, cosmetics, and food industries, enzymes are used to speed up, regulate, or control processes to obtain a valuable final product. They are used in extracting fruit juice, tanning leather, the production of biofuel and biopolymers, insulin in pharmaceuticals, and growth hormones [34,35]. Yeast and *Escherichia coli*, through the biotechnological processes of transformation and functional metagenomics etc., have undergone several genetic manipulations to express various extremozymes of interest [36–38]. Extremozymes from marine microorganisms have been applied in bioethanol production. An example is the case of alpha-amylase from *Thermococcus* spp., a microorganism from deep sea hydrothermal vents: the extremozyme is commercially available as Fuelzyme [9]. *Zunongwangia profunda* (MCCC 1A01486), a marine bacterium, produced an alpha amylase that hydrolyzes 1–4-linked glucans in starch [38]. *Aeromonas* sp. *EBB-1, Candida antarctica*, and *Photobacterium lipolyticum* have been identified as marine microorganisms that produce lipase used for biodiesel production [9]. *Bacillus safensis* (CFA-06) is a bacterium isolated from the Campos Basin in Brazil and is found to produce an oxidoreductase proven to have relevant applications in the biodegradation of aromatic compounds [9,39].

The detergent industry is the largest market for enzyme production in the world [40,41]. All types of detergents, ranging from those produced for household laundering to reagents used for cleaning of contact lenses, use proteases as a standard ingredient [41]. Amylases from marine microorganisms have been used in detergent-producing industries to enhance the ability of detergents to remove tough stains. Alpha-amylase from *Bacillus* strains is used for desizing processes in textile industries. In paper making industries, amylase is used for the modification of starch for paper coating. This modification helps to improve the quality of writing on paper by making the surface of paper smooth and strong [12]. The increased solubility of substrate and the lower substrate viscosity are the major reasons why enzymes with high thermostability are very active at higher operating temperatures.

In the medical and pharmaceutical industries, some marine enzymes have been identified to exhibit anticancer activity by means of nutrient depletion, anti-angiogenesis, or the induction of apoptosis. The treatment of cancer poses a serious challenge to the medical and pharmaceutical sciences. The challenges associated with the inability of chemotherapies to differentiate between tumor cells and healthy cells, coupled with the issue of drug resistance, has resulted in poor chemotherapeutic outcomes [31]. Research has proven that biomolecules such as enzymes from marine microorganisms are more cytotoxic than their terrestrial counterparts [15,42]. The auxotrophic nature of cancer cells is the rationale behind the enzymatic therapy approach.

5. Current Issues on Sponge–Microbial Symbiosis and Marine Extremozyme Application

5.1. Research

There are challenges in the research of enzymes from marine extremophiles. We have observed from the cascade of publications in this subject that there is much to be discovered from marine extremophiles with regards to the wealth of the habitat [9,43]. Regardless of the acknowledgement of many scientists about the potential of marine microorganisms, very few studies have sampled sponges for comparison with other samples such as marine water, sediment, and hydrothermal vent, etc. [9,43]. The bulk of research with marine sponge as samples has not reported the enzymatic activities or potential of the symbiotic bacteria in sponges. Besides, a good number of the published research on marine microorganisms have been performed in mangroves and coastal areas. This challenge facing research is tied to the fact that accessing the deep sea with the required technology is still quite expensive and challenging [9,43]. The cost of sampling the sea with a well-equipped research ship is within the range of USD 20,000–30,000 per day [2]. There is a great deal of information and many publications on bacteria inhabiting the deep sea. However, the opposite is the case when it comes to fungal inhabitants of such extreme environment. [44]. Many microbiologists are faced with the challenge of the low cultivability of marine microorganisms in conventional laboratory culture media. This method is no longer reliable or sufficient for the study of marine extremophiles as approximately 99% of them cannot be cultured on conventional culture media at present [9,38,43,45–48]. Significantly low percentages (0.001–1%) of the isolated samples are culturable or enumerated on growth media or recovery media [49]. The reasons for the difficulties experienced in the development of the majority of microbial cells in laboratory-based culture media include:

a. The possibility of distortion or destruction of microbial interactions in their natural environment by culture media. The rapidly growing microbes may produce inhibitory compounds that will in turn inactivate the very slow growers.
b. A lack of knowledge of organic substrates that are metabolized by the marine microorganism in their natural habitat may have resulted in the wrong concentration or absence of nutrients in the available culture media.
c. The possibility of virus infection may prevent bacterial growth in medium; this could be due to infection with bacteriophages or starvation of bacterial cells due to changes to the lytic cycle of temperate phages during the supply of nutrients.
d. Difficulty in the detection of growth may also be associated with high concentrations of substrate in the medium. This condition may be toxic to bacteria that have evolved under oligotrophic conditions.
e. Poor cell density detection methods have resulted in negligence regarding the invisible first round of culture in liquid media [49].

Finally, it has been discovered that, during sampling, many researchers approach the field (marine environment) blindly. Very few conduct remote sensing before deciding to approach a sampling site [9]. The implication is a reduction in the certainty that the desired extremozyme will be present. However, a blind approach to researching an environment or site is not out of place if the occasion warrants it. Against such backdrop, the researcher increases the risk of wasting resources if the research aims and objectives are very specific for certain extremozymes of interest [9].

5.2. Industry and Bioremediation

As earlier stated, the unique properties of marine extremozymes are the driving force behind their application in biotechnological processes and in the solving of other problems [2]. Unfortunately, very few marine extremozymes are utilized for industrial and biotechnological purposes because of the problem of cost-effective, large-scale production for commercialization [47]. In 2020, a detergent additive lipase enzyme produced from agricultural waste was developed, but the cost of adopting a biotechnological approach to mass production remains a challenge, despite the global acknowledgement of such category of lipase [50–52]. Enzymes are now commonly used for the formulation of detergents in many countries. However, the details of the specific microorganisms from which to source the enzymes with the desired properties and qualities still present a challenge [30]. Many detergents in developing countries in Africa and Asia are derived from petroleum. Such practice may over time result in a shortage of raw materials since crude oil is a nonrenewable natural resource and lead to the problem of environmental pollution from the disposed waste, which cannot readily be broken down.

Through modern biotechnology, bacterial and fungal isolates from marine habitats are now used in the bioremediation of colored wastewater. Marine microbial enzymes have a significant ability to degrade and decolorize azo dyes obtained from industrial wastes. The fungal extracellular enzymes lignin peroxidase, lacase, and manganese peroxidase, have been used to partially degrade lignin and chlorinated phenolic compounds from paper and pulp industries. Alkaline xylanase and thermostable metal-tolerant enzymes from *Aspergillus niger* and *Cerrena unicolor* from marine water have been reported to be effective in the bioremediation of industrial wastes [53].

6. Prospects

6.1. Cost Effectiveness

There is an urgent need for cost-effective methods of assessing and processing samples from the deep sea. It is a major panacea to the problem of limited marine extremozymes with outstanding potential for industrial and biotechnological applications. In addition, cost-effective strategies will also provide a bearing for the cost-effective, large-scale production of extremozymes for industrial and biotechnological utilization [47,52].

6.2. Species Diversity Approach

Species diversification approach and taking advantage of species interrelationships, especially in extreme environments, could be promising in the search for extremozymes. The bioremediation of hazardous substances in large waterbodies, could be more effective if more attention is given to the biocatalytic exploration of enzymes from sponge–microbial symbiosis in extreme marine habitats, as a huge variety of industrial wastes are released into the marine environment every day [1,6,33,53]. The limited information on fungal extremophiles in the deep sea compared to their bacterial counterparts represents an imbalance that needs immediate attention. Fungal microorganisms could be of potential of interest for industrial and biotechnological applications. For the purpose of providing solutions to the demands of enzyme production, the degradation of xenobiotics, and tackling the problems of bioremediation, a fungal survey in extreme environments, such as the hypoxic and hydrothermal vents [44], should be initiated. Bacterial and fungal microorganisms have been useful in the bioremediation of pollutants. The laboratory reports of halotolerant fungi are quite encouraging [54,55]. There is need to improve applications for the use of halotolerant fungi and enzymes in solving problems of bioremediation, food, industry, and biotechnology at large [44].

6.3. Integrative Bioinformatics Software

The practice of metagenomics has provided a solution to the study of over 98% of microorganisms that cannot be cultivated in bioactive-based compounds in our laboratories [33]. It is a powerful biotechnological tool for exploitation of bioactive molecules such as enzymes from marine microorganisms, a combination of marine biotechnology and metagenomics with high-throughput screening techniques [33]. Furthermore, despite the recent developments in bioinformatics software, there is still need for integrative bioinformatics software for meta and mega genomics [47,48].

6.4. Government and Industries

Could the quest for enzymes with more novel properties be satisfied? Governments, institutions, multinational industries, and companies need to support research associated with marine microbial enzymology. Since this is an emerging area, only very few marine extremozymes are utilized for industrial and biotechnological purposes [43]. Therefore, efforts should be made to ensure the availability and affordability of enzymes with unique physiological properties that are highly fit for industrial and biotechnological application [34].

7. Conclusions

The use of marine extremozymes spans many industries. Their results in food processing, cancer studies, paper production, detergent production, etc., are very promising. There is no doubt that the unique properties of marine extremozymes are the driving force behind their application in industrial and biotechnological processes. Species diversification approaches and taking advantage of species interrelationships, such as sponge–microbe symbiosis, are very promising for extremozymes. There is need for the development of cost-effective methods supported by governments and industry. The use of remote sensing should be incorporated into sampling techniques for research studies that sample extreme environments. An integrative bioinformatics software for metagenomic studies of marine microorganisms should be developed.

Author Contributions: Conceptualization, P.T.N. and R.U.C.-O.; methodology, All authors; software, P.T.N.; validation, All authors; writing—original draft preparation; P.T.N.; writing—review and editing, P.T.N., E.A. and R.U.C.-O.; supervision, H.R.M., E.A. and R.U.C.-O.; project administration, P.T.N.; funding acquisition, R.U.C.-O. and P.T.N. All authors have read and agreed to the published version of the manuscript.

Funding: This research is funded by the University of the West of England (UWE) Bristol United Kingdom and The African Investment Gateway Group London United Kingdom. Grant code: RDAS0163.

Data Availability Statement: Not applicable.

Conflicts of Interest: The authors declare no conflict of interest.

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