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Potential impact of Andrassy bentonite microbial diversity in the long-term performance of a deep nuclear waste repository

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Abstract. Copper and steel canning and bentonite buffer are normally forseen as the primary containment component of a deep nuclear waste repository. Distribution of microbes in subsurface environments have been found to be extensive and directly or indirectly may exert influence on waste canister corrosion and the mobility of radionuclides. The understanding of clays and microbial interaction with radionuclides will be useful in predicting the microbial impacts on the performance of the waste repositories. The present work characterizes the culture-dependent microbial diversity of Andrassy bentonite recovered from Tawau clay deposits. The evaluation of microbial populations shows the presence of a number of cultivable microbes (e.g. *Staphylococcus, Micrococcus, Achromobacter, Bacillus, Paecilomyces, Trichoderma,* and *Fusarium*). Additionally, a pigmented yeast strain *Rhodotorula mucilaginosa* was also recovered from the formation. Both *Bacillus* and *Rhodotorula mucilaginosa* have high tolerance towards U radiation and toxicity. The presence of Rhodotorula mucilaginosa in Andrassy bentonite might be able to change the speciation of radionuclides (e.g. uranium) in a future deep repository. However, concern over the presence of Fe (III) reduction microbes such as Bacillus also found in the formation could lead to corrosion of copper steel canister and affect the overall performance of the containment system.

1. Introduction

Malaysia has interest in utilizing nuclear energy in the near future as a potential alternative for long-term energy production in peninsular Malaysia under the Economic Transformation Programme (ETP) [1][2]. The Malaysian Nuclear Power Corporation (MNPC) has been entrusted to undertake a comprehensive study to facilitate the Malaysian Government in the planning of nuclear power program including a proper disposal of high-level nuclear waste (HLW) to ensure better and safer environment [3]. It is expected that nuclear wastes generated in the nuclear fuel cycle must be safely stored for at least 100,000 years for the radiotoxicity to decrease to the levels similar to those of natural uranium. Countries such as Sweden, Finland and France have opted for deep geological repositories (DGRs) as ultimate disposal of HLWs that is expected to be fully functional by the year 2020 [1].

The structure of DGR utilizes bentonitic clay as the buffer material. Bentonite has been selected due to its uniquely low permeability, self-sealing membrane characteristic, durability and high sorption capacity, making it a viable buffer material for HLW [4][5]. As of today, the sodium-based MX80 bentonite has been extensively researched and considered as international reference material for DGRs [5][6]. On the other hand, Deponit CA-N bentonite, from Milos formation has been considered as European standard [7][8]. Based on the these reports, various countries around the world have established their own reference material for example FEBEX for Spain, Kunigel V1 for Japan, GMZ for China to name a few [4][9][10][11]. Subsequent to national decision on nuclear power, Malaysia needs to consider its own reference material for development of its own DGR facility.

A typical bentonite is highly swelling clay derived from volcanic formation occurred several thousand years following the Pliocene age [12]. In Malaysia however, most of the bentonite used in the country are imported from neighboring country such as Pakistan and Indonesia [13]. Commercial usage of locally available bentonite was found to be scarce or nonexistent. The deposit sits in the geological heritage of Sabah and is well preserved. The first occurrence of bentonite formation in Malaysia was reported by Irawan and Samsuri [14] in the volcanic formation in Andrassy, Sabah [15]. Study of this material is crucial for the development of Malaysia's nuclear power disposal programme and may be considered as Malaysia's reference material, however, detailed characteristics and behaviour of Andrassy bentonite are scarcely documented. To date previous studies only focused on geotechnical and microbiological characterization of Andrassy bentonite and their effect on the water retention characteristics [10][16][17].

The safety of this long-term geological disposal could be compromised by physical and chemical factors, and also by the biogeochemical activity. Thus, the microbiological interactions between bentonite buffer material, host rock and copper steel canister plays a significant role in the long-term stability of the DGR. Microbiological processes can affect the geochemistry of bentonite barriers through four different mechanisms: (i) reduction of structural Fe (III) of clay minerals, (ii) alteration of mineral surfaces by the production of siderophores and small-organic acids, (iii) formation of biofilm in the clay mineral surface and (iv) to control the speciation and mobility of radionuclides [18]. For instance, previous studies have shown that the presence of *Geobactor Sp.* in MX80 bentonite has the ability to degrade radioactive material contamination [19]. On the other hand, the presence of *Rhodotula Sp.* found in Spain bentonite has the ability to precipitate Uranium based radionuclides [18]. To the best of the authors' knowledge, no attempt has been made to study the influence and potential of Andrassy bentonite microbiological diversity on the long-term performance of DGR.

The objective of the present work is twofold (i) to identify the microbiological characteristics of Andrassy bentonite and (ii) to determine the potential impact of the microbes identified on both corrosion of copper steel canister and interaction with radionuclides.

2. Material and Methods

2.1. Geographical description of the clay sample recovery sites

The bentonite samples used in this work were recovered from Andrassy in Tawau (N4º 18.97'- E 117º 57.37') shown in Figure 1. Sample was first excavated and characterized by determining the physical, chemical mineralogical and microbiological properties. The sample was excavated on site by using a hand auger up to a depth of about 5 meter. The soil sample were carefully sealed and placed in an air-tight container to avoid contamination. The geotechnical properties is presented in Table 1.

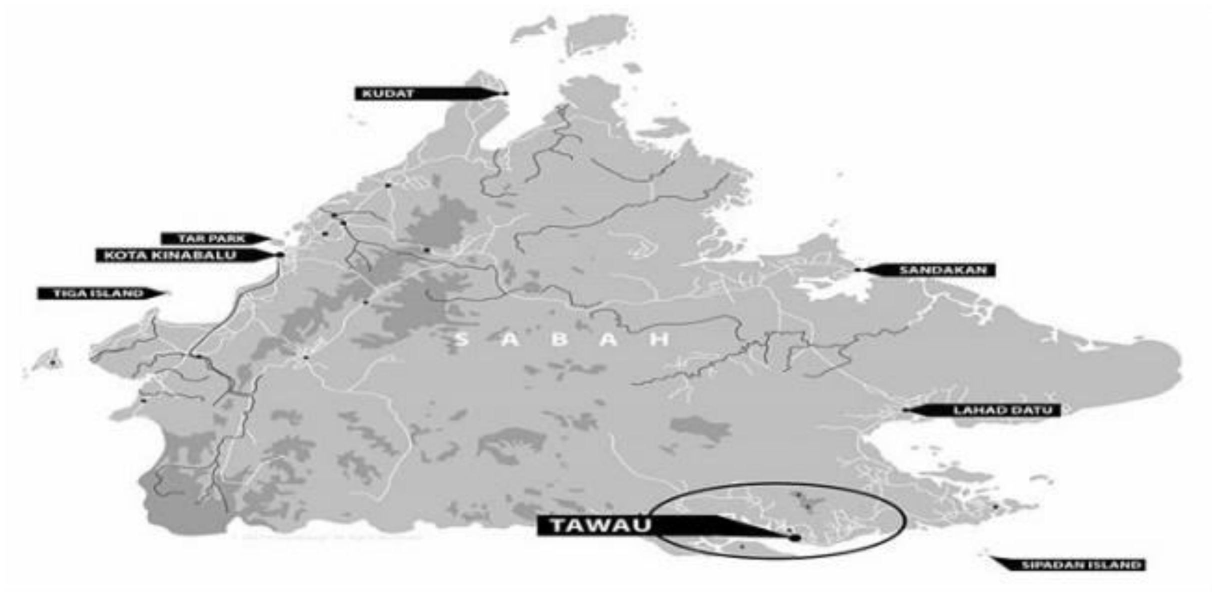

Figure 1. Map of Andrassy bentonite sample locations.

Table 1. Comparison of Geotechnical properties of Andrassy bentonite with international reference bentonites [10][17]

Properties	Andrassy	MX80	Deponit CA-N	GMZ
Specific gravity, Gs	2.78	2.80	2.84	2.66
Liquid limit (%)	129.30	437	135	276
Plastic Limit (%)	46.12	63	58	37
Shrinkage limit (%)	13.40	12.2	13.6	-
Cation exchange capacity (meq/100g)	42.15	90.31	42.78	77.3
Specific surface area (m^2/g)	734.27	676	734	570
Main minerals (%)				
Montmorillonite	63.20	75.00	81.00	75.4
Quartz	11.50	10	-	-
Vermiculite	25.30	-	-	-
Graphite	-	-	19	-
Cristobalite	-	15	-	-

2.2. Determination of microbiological characteristics

The microbiological properties of the bentonite, namely bacteria and fungus determination were carried out following plating, slide culture, streaking and isolation techniques [20]. Potato dextrose agar (PDA) was used for culturing fungi, whereas Nutrient agar (NA) was used to culture bacteria. The bentonite specimen was initially suspended in 0.9% NaCl solution to separate the microbes [21]. Identification of the specific strain of each microbes after isolation was carried out in an independent laboratory using polymerase chain reaction (PCR) protocol and referred to international microbiological characterization database.

3. Results and discussion

The bentonite in this study was found to exhibit a large surface area and high surface charge characteristics which make it ideal for soil microbes [16][17]. Based on the plating, isolation and PCR techniques, the microbes determined in Andrassy bentonite is presented in Table 2. Concurrent with the large surface area, nine microbes were successfully isolated and identified. These isolates belonged to the phyla Firmicutes, Actinobacteria, Proteobacteria, Sac Fungi, and Mycota. Based on origin of closed related sequence (i.e. where these types microbes can be abundantly be found), six of them were commonly found in soil. Surprisingly, two bacteria most commonly found in tobacco leaf and textile effluents are also present. Three species of bacteria (i.e. *Bacillus anthracis, Staphylococcus aureus, Micrococcus luteus*) and one species of fungus, a yeast strain (i.e *Rhodotorula mucilaginosa*) found in this study was similar to that of bacteria and fungi found in bentonite from Spain [18]. Another type of microbe identified was *Escherichia coli*. However, this bacteria was ruled out due to possible cross contamination during handling of the soil specimens during transport and preparation [16].

Table 2. The microbiological properties of Andrassy Bentonite

Phylum	Closest phylogenetic relative	Origin of closed related sequence
Bacteria		
Firmicutes	*Bacillus anthracis*	Soil
Firmicutes	*Staphylococcus aureus*	Tobacco leaf
Actinobacteria	*Micrococcus luteus*	Textile effluent
Proteobacteria	*Achromobacter xylosoxidans*	Soil
Proteobacteria	*Escherichia coli*	Fecal matter
Fungus		
Sac Fungi	*Paecilomyces lilacinus*	Soil
Ascomycota	*Trichoderma atroviride*	Soil
Sac Fungi	*Fusarium proliferatum*	Plant and soil
Basidiomycota (Yeast)	*Rhodotorula mucilaginosa*	Soil

3.1. Interactions of Andrassy bentonite microbial populations on radionuclides

In the case of radionuclides spillage or leakage from the DGR's gallery, the bentonite buffer would serve as buffer medium to prevent the migration of radionuclides. Furthermore, the microbes available in the buffer material would react to the movement of the radionuclides. The yeast strain *Rhodotorula mucilaginosa* found in the bentonite samples have high tolerance towards high concentration of Uranium. The yeast strain is also capable of precipitating Uranium based radionuclide into less hazardous U-phosphate minerals phases [18]. Thus, the presence of *Mucilaginosa R.* is crucial for bioremediation of radioactive contaminated sites including DGR applications. In addition, the presence of *Bacillus Sp*. have been shown to convert aerobic uranium (VI) precipitation to insoluble U phosphate phases due to the activity of acidic phosphatase which in turn assisted in immobilizing different radionuclides [22].

3.2. Effect of Andrassy bentonite microbial populations on corrosion of HLW's canister

The copper steel canister would be placed in close vicinity to the bentonite buffer material. Thus, interactions between microbes found in the bentonite buffer and the canister is expected to occur. Apart from beneficial microbes explained in Section 3.1, the presence of other Fe and Cu reducing microbes found in the same bentonite specimen would cause corrosion problems to the copper steel canisters used for storing HLWs. For instance, the presence of *Mucilaginosa R.*has also been reported to cause damages to aircrafts [23]. They can cause degradation of aircraft components due to their ability to uptake Cu [24]. Similarly, the existence of *Luteus M., Aureus S., Xyloxidan A.* and *Lilacinus P.* may also contribute to the degradation of the canister under long exposures. Studies revealed that these microbes have the ability to cause corrosion in medical devices, steel pipelines and transmission towers [25][26][27]. Due to their unique ability, these microbes has long been used as remediation agents in remediation of contaminated sites and contaminated water all over the world [28][29][30]. However, in the perspective of construction of DGRs, in the long-term these microbes may degrade and affect the stability of the copper steel canister and causes leakage of radionuclides into the environment.

4. Conclusion

The present study describes the culture-dependent microbial diversity of Andrassy bentonite formations. The bentonite were carefully collected and characterised. Based on the microbiological analyses, it was noted that the bentonite contained beneficial microbes (i.e. *Mucilaginosa R.* and *Bacillus Sp.*) for DGR application as buffer material in restricting the migration of radionuclides. On the contrary the bentonite also consists of microbes (i.e. *Luteus M., Aureus S., Xyloxidan A.* and *Lilacinus P.)* that have to potential to induce corrosion to copper steel canister used for safe long-term storage of the HLWs.

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