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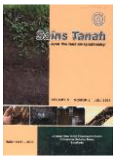

Re-evaluating the evidence of aflatoxin B1 intercalation into smectite interlayer: a review based on basal spacing data

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ABSTRACT

The bonding mechanism of aflatoxin B_1 (AfB_1) onto smectite in the batch adsorption test remains a subject of ongoing debate. One key aspect under contention is whether the interlayer of smectite is accessible to the AfB_1 molecule. Some researchers contested the possibility of AfB_1 intercalation into the smectite interlayer by highlighting the lack of discernible differences in basal spacing value before and after the adsorption experiment. Conversely, others have advanced the opposite argument based on observations indicating an increase in basal spacing value after adsorption. This study aims to resolve the discrepancy through a comparative examination of data from several previous studies that reported basal spacing values derived from X-ray diffraction (XRD) analysis, both before and after adsorption tests. By also considering the characteristic swelling behavior of smectite, this comparative examination explains the differing perspectives. The lack of discernible differences in basal spacing value pre- and post-adsorption tests, which was interpreted as the absence of AfB_1 intercalation, was attributed to the fact that the initial samples were inherently in hydrated condition, as indicated by basal spacing values of 1.41 to 1.59 nm, whereas in the dry state, these values would typically be ~0.98 to ~1.0 nm, thereby precluding further expansion. Consistent with a previous study that suggested AfB_1 bonding to smectite in hydrated conditions occurred through hydrogen bonding within the interlayer, we propose that the hydration state of smectite will significantly influence AfB_1 uptake. Thus, further research of adsorption test to investigate the relationship between AfB_1 uptake as a function of the hydration state on smectite minerals is strongly suggested.

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1. INTRODUCTION

Numerous strategies have been developed to address aflatoxin B_1 (AfB_1) contamination, encompassing biological, chemical, and physical approaches (Cai et al., 2025; Savić et al., 2020; H.-B. Wang et al., 2023; B. Yang et al., 2022). Biological methods, such as bio-pesticides, bacteria, and yeasts, have demonstrated significant results in controlling aflatoxins. Bio-pesticides, usually applied in agricultural fields, are derived from atoxigenic strains that compete with toxigenic strains of *Aspergillus flavus*, thereby suppressing the growth and reproduction of aflatoxins (Abbas et al., 2017). Among the various bacteria, *Bacillus* spp. has been the most broadly investigated for its potential in reducing aflatoxin. *Bacillus subtilis* has been reported to effectively inhibit the growth of *Aspergillus parasiticus* by up to 92% and the production of aflatoxin by up to 100% (Siahmoshteh et al., 2018). In experimental studies, several yeasts strains such as *Debaryomyces hansenii* strain BCS003, *D. hansenii*, *Kluyveromyces* spp., *Pichia anomala*, *Candida maltose*, *Saccharomyces cerevisiae* RC008, and *Saccharomyces cerevisiae* RC016, have exhibited significant inhibitory effects

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on aflatoxin production and *Aspergillus* spp. growth as well (Ren et al., 2020). For chemical control, several studies reported the use of ozone and chitosan nanoparticles for aflatoxin reduction (Sipos et al., 2021). Ozone detoxifies aflatoxin by destroying the double bond of the furan ring through electrophilic attack, thereby converting it into non-toxic compounds such as aldehydes and acids (Atakan & Caner, 2021). Chitosan, at alkaline pH, has positively charged groups that enable it to bind with negatively charged aflatoxin (Ghobish et al., 2025; Hernandez-Patlan et al., 2018). Physical approaches such as thermal treatments, irradiation, and mechanical sorting, have been applied to decrease aflatoxin contamination in food commodities (Shabeer et al., 2022). Although aflatoxins are heat-resistant, methods such as roasting, boiling, and autoclaving can considerably degrade their levels, depending on the circumstances and commodity type (Shabeer et al., 2022). Non-thermal techniques, including cold plasma and gamma irradiation, have demonstrated efficacy rates of up to 95% and 97%, respectively (Shabeer et al., 2022; Sipos et al., 2021). Additionally, adsorbents like activated carbon, montmorillonite clay, diatomite, talc, and zeolite showed satisfactory aflatoxin-binding efficiencies of 93.5%, 86%-100%, 90%-100%, 60%-100%, and 70%, respectively (Jiang et al., 2023; Oladele et al., 2025; Sprynskyy et al., 2018; Zavala-Franco et al., 2018).

Smectite, a group of expandable 2:1 phyllosilicate clay minerals, has been utilized as one of the strategies to remove AfB_1 from aqueous systems since the late 1970s (Masimango et al., 1978). Several researchers have reported that bentonite showed excellent performance in removing AfB_1 from aqueous solution (de Lima Schlösser et al., 2024; Gan et al., 2019; Nones et al., 2017; Wang et al., 2020; Wongtangtintan et al., 2016; Zhao et al., 2022). However, researchers have disagreed on the precise bonding mechanism between AfB_1 and smectite. Various perspectives proposed by researchers include the electron donor-acceptor model, which suggested an interaction between the carbon atom in the carbonyl of AfB_1 and the negatively charged surface of the smectite mineral layer (Phillips, 1999; Phillips et al., 2008); hydrogen bonding interaction between the double hydrogen atoms in the furan ring of AfB_1 and the edge of smectite(Desheng et al., 2005); epoxidation of double-bonded carbons on the furan rings of AfB_1 by the oxygens of the interlayer smectite surface (Tenorio Arvide et al., 2008); hydrogen bonding between the two carbonyl groups of AfB_1 and water molecules hydrating the interlayer cations in wet conditions, and ion-dipole interactions between the two carbonyl groups of AfB_1 and the interlayer cations in dry conditions (Deng et al., 2010); simultaneous binding of released cations from the interlayer, which then adhered to the four oxygen atoms of the Si-O ring on the smectite mineral layer surface, forming a strong bridging-linkage $(C=O)_2{\cdot\cdot}Ca^{2+}{\cdot\cdot}(O\text{-}Si)_4$ (Kang et al., 2016); and ion-dipole interaction of the two carbonyl groups with the cations in the edge smectite layer and the outermost interlayer cations (Aisawa et al., 2018). On the other hand, some of the researchers emphasized that bonding occurred only at the outer or edge of the smectite layer (Aisawa et al., 2018; Desheng et al., 2005), while others suggested that AfB_1 was able to penetrate the interlayer (Deng et al., 2012; Deng et al., 2010; Kannewischer et al., 2006; Phillips, 1999).

The objective of this study is to address the existing discrepancies within the literature pertaining to AfB_1 intercalation into smectite interlayer through a critical analysis of X-ray diffraction (XRD) basal spacing data from prior studies. A synthesis of the divergent perspectives from the dissenting researchers is conducted, drawing insights from the d_{001}-value data analysis before and after adsorption tests, as reported in their respective papers. The interlayer of smectite is a crucial region as it accommodates the adsorbate binding site. A larger basal spacing typically enhances the entrapment of the adsorbate molecule. This study holds significance as it will provide new insight into the types of smectite exhibiting high adsorption capacity, underpin the future development of modifications to improve the adsorption performance, and contribute to the establishment of molecular and adsorption isotherm models for AfB_1. We have identified four relevant articles presenting contradictory interpretations based on d_{001}-value data. Before delving into the discussion on the bonding mechanism of AfB_1 on smectite, the properties of AfB_1 and smectite mineral are briefly outlined.

2. AFLATOXIN B_1

Aflatoxins are a group of toxic compounds produced by fungi, especially *Aspergillus flavus* and *Aspergillus parasiticus* (Abrehame et al., 2023; Okechukwu et al., 2024; Shabeer et al., 2022). Aflatoxins are commonly detected as contaminants in various foods such as rice (Ali, 2019; Katsurayama et al., 2018; Naeem et al., 2024), peanuts (Asare Bediako et al., 2019; Krska et al., 2022; Masaka et al., 2022), maize (Falade et al., 2022; Kamika et al., 2016; Massomo, 2020), dried fruits (González-Curbelo & Kabak, 2023; Heshmati et al., 2017; Naeem et al., 2022), cereals (Kousar et al., 2024; Kumar et al., 2022; Wei et al., 2024), cooking oil (Abbas et al., 2017; Karunarathna et al., 2019; Kholif et al., 2022; Waqas et al., 2021), particularly in post-harvest products exposed to high relative humidity or wet condition (Gómez-Salazar et al., 2023; Mutuli et al., 2022). There are more than 20 types of aflatoxins have been identified, but the most concerning are: B_1, B_2, G_1, and G_2 (Ji & Xie, 2020; Popescu et al., 2022; Wang et al., 2022). Among these, AfB_1 is the most harmful and potent, posing a serious threat to human and animal health due to its carcinogenic properties (Adam et al., 2022; Marchese et al., 2018; Mutocheluh & Narkwa, 2022; Nugraha et al., 2018). The term "A-fla-toxin" is derived from *A.flavus*, with the "B" in AfB_1 indicating its blue fluorescence (Dhakal et al., 2025; Lalah, 2019). It has been reported that AfB_1 is strongly suspected to cause up to 28% of all cases in the world of hepatocellular carcinoma (HCC), the predominant form of liver cancer (Liu et al., 2017). Individuals who are hepatitis B virus (HBV) positive and contract AfB_1 are estimated to face a 30 times higher risk of developing liver cancer compared to those who are HBV negative (Do et al., 2020). In addition, studies have documented adverse effects of AfB_1 on poultry, including liver pathological changes (Y. Wang et al., 2023), inflammatory damage, immunosuppression, and inhibition of

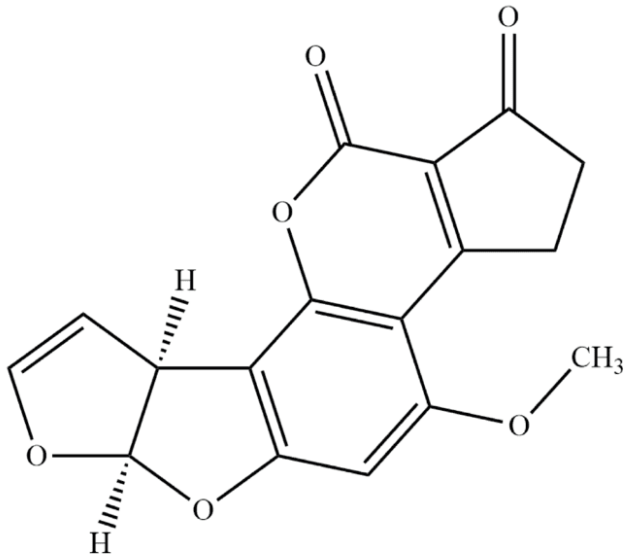

Figure 1. Chemical structure of AfB_1 (drawn using Marvin 17.21.0, Chemaxon, based on structural data from PubChem (https://pubchem.ncbi.nlm.nih.gov/) (accessed on April 25, 2024)

chick growth (Hou et al., 2022). Alarmingly, despite aflatoxins primarily accumulating in plant tissue, their contamination easily spreads to soil and water systems through rainfall (Juraschek et al., 2022). This insidious route leads to a high potential risk of exposure, particularly for individuals reliant on these water sources for drinking purposes.

AfB_1 is composed of five interconnected rings (Fig. 1), namely dihydrofuran, furan, methoxyphenyl, δ-lactone, and cyclopentenone (Phillips, 1999). Deng and Szczerba (2011) reported based on computed energy, net atomic distribution, and molecular dynamics simulation, consistently indicated that the oxygen atoms of the carbonyl groups at δ-lactone and cyclopentenone rings played a primary role in attracting positively charged species. The energy calculation after optimizing the AfB_1 configuration revealed that the lowest energy in the attraction of two cations, Na^+ and Mn^{2+}, with the oxygen atoms on two carbonyl groups was in the region between these carbonyl groups. At equilibrium condition, over 96% of interaction between AfB_1 and cations occurred within this region of the two carbonyl groups compared to other regions. The surface electrostatic potential at the two carbonyl groups exhibited the most negative values, while the methyl (CH_3) group area displayed the most positive surface electrostatic potential. Interactions involving the oxygen atoms of the dihydrofuran ring and the lactone ring were found to be non-significant (Deng & Szczerba, 2011).

3. SMECTITE

3.1. Bentonite And Smectite

The term "bentonite" was first introduced in 1898 by Knight to describe a specific type of clay exhibiting characteristic properties such as soapy texture, plasticity, and high colloidal nature, discovered in a Cretaceous-age bed near Fort Benton. Several definitions have been proposed after Knight's definition, however, the term bentonite is lately clearly defined as any clay containing predominantly smectite mineral whose physical properties are dictated by this mineral (Grim & Guven, 1978). Montmorillonite is the most common type of smectite, characterized by a structure comprising one octahedral sheet sandwiched between two tetrahedral silica sheets, commonly referred to as a 2:1 type (Fig. 2). These smectite layers are vertically stacked along the c-axis direction. The space between layers, known as the interlayer, is bonded by van der Waals and electrostatic forces, typically involving interlayer cations such as Na^+ or Ca^{2+}. Although the bonds within the interlayer are weak, they exhibit excellent cleavage. The interlayer is accessible for water molecules or other polar molecules, allowing for swelling in the c-direction. The distance between the uppermost tetrahedral sheet of two neighboring smectite layers within a stack is referred to as the basal spacing. By analyzing the intensity and position of the 001 reflection in XRD, the length of the basal spacing can be determined. Basal spacing is an important factor in the characterization of smectite minerals, as the change in its values can indicate hydration state alteration, the presence of molecules within the interlayer, and other structural modifications.

3.2. Source Of Smectite Charge

The penetration of cations into the interlayer is due to the insufficiency of charge on the surface of the smectite mineral layer, resulting from isomorphic substitution. Isomorphic substitution is a replacement of the central cation in the tetrahedral or octahedral sheet by another cation of almost the same size without changing the crystal structure (Fig. 3).

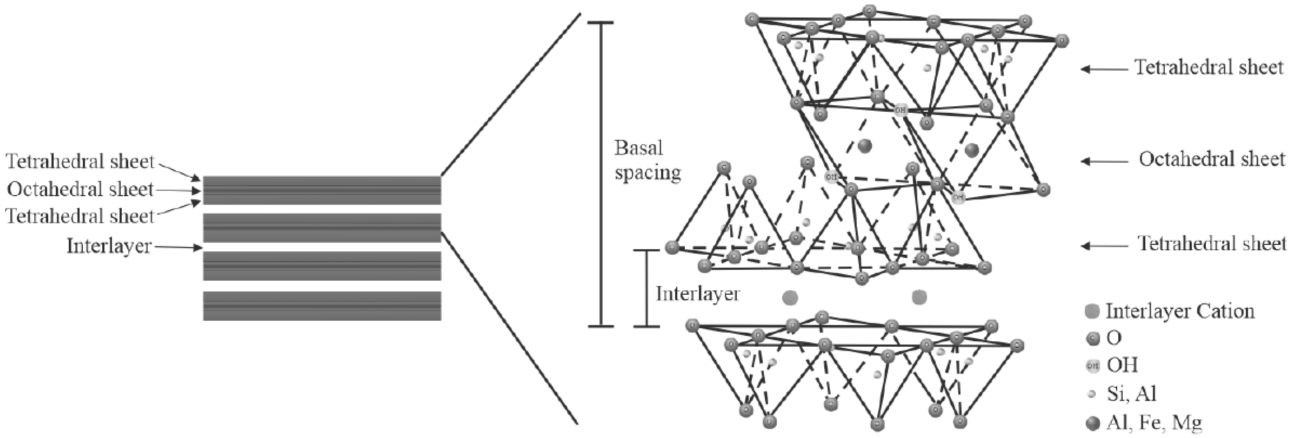

Figure 2. Structure of smectite mineral (modified from Grim and Guven (1978))

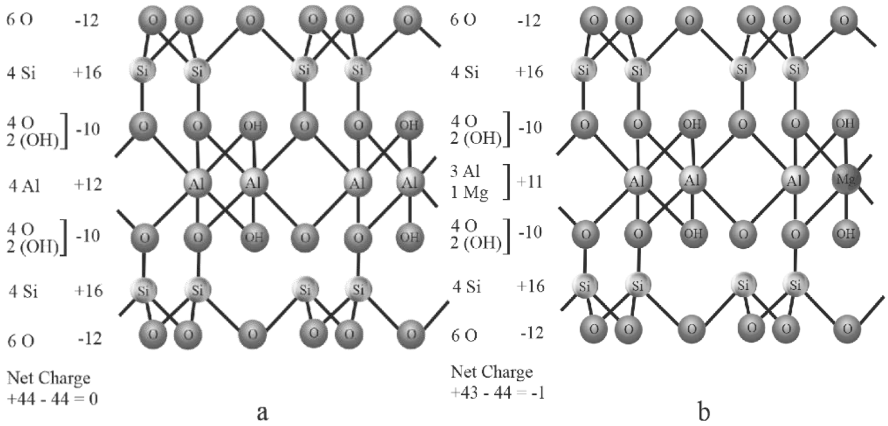

Figure 3. a. Illustration of smectite mineral before isomorphic substitution, demonstrating a neutral charge. b. Illustration of smectite mineral after isomorphic substitution, exhibiting a charge deficiency due to substitution of Al^{3+} by Mg^{2+}. This isomorphic substitution type leads to the formation of montmorillonite species (modified from Essington (2003))

Table 1. Species nomenclature of smectite group according to cation substitution (Marshall (1964); Warshaw and Roy (1961); Ross and Hendricks (1945))

Type	Group	Subgroup	Species	Tetrahedral sheet substitution	Octahedral sheet substitution
2:1	Smectite	Dioctahedral smectite	Montmorillonite	None	1 Mg^{2+} for every sixth Al^{+3}
			Beidellite	Al^{+3} for Si^{4+}	None
			Nontronite	Al^{+3} for Si^{4+}	Fe^{+3} for Al^{+3}
		Trioctahedral smectite	Hectorite	None	Li^{+} for Mg^{2+}
			Saponite	Al^{+3} for Si^{4+}	Fe^{3+} for Mg^{2+}
			Sauconite	Al^{+3} for Si^{4+}	Zn^{+2} for Mg^{2+}

For instance, Si^{4+} is substituted by Al^{3+} in tetrahedral coordination. Al^{3+} is substituted by Mg^{2+}, Fe^{2+}, or Fe^{3+} in octahedral coordination. The type of cation and its substitution location, whether in tetrahedral or octahedral coordination, determine the species name of the smectite, as outlined in Table 1. This substitution generates a negative permanent charge if the replacement cation has a lower charge, thereby attracting other cations into the interlayer space via electrostatic forces. Isomorphic substitution is a natural occurrence during the formation of minerals.

3.3. Swelling Behavior of Smectite

Smectite has a hydrophilic property which adsorbs water molecules into its interlayer due to cations hydration, resulting in basal spacing expansion. Although water molecule is electrically neutral, it possesses polarity because the oxygen atom exhibits higher electronegativity compared to the hydrogen atom, causing the oxygen atom to attract the shared electrons more strongly than the hydrogen. Consequently, this unequal sharing of electrons generates partial negative charge on the oxygen atom and partial positive charge on the hydrogen atom. In the hydration process of smectite, the negative dipoles of water molecule align toward the interlayer cations, weakening the electrostatic interaction between the negatively charged surface of the smectite mineral layers and the interlayer cations (Madsen & Müller-Vonmoos, 1989). The expansion of basal spacing due to the hydration follows a stepwise mechanism corresponding to the increase in the number of discrete sheets of water molecules in the interlayer (Chávez-Páez et al., 2001; Madsen & Müller-Vonmoos, 1989; Tamura et al., 2000; Zhang et al., 2016). In the case of Na- and Ca-smectite montmorillonite, interlayer hydration results in the arrangement of 1, 2, 3 layer-hydrates, corresponding to 32, 64, 96 water molecules, respectively, with each new layer forming after the preceding layer is completely formed (Zhang et al., 2016). The interlayer expansion at 0, 1, 2, 3 layer-hydrates is reported as 0.899, 1.153, 1.458, 1.704 nm for Na- smectite montmorillonite, and 0.974, 1.224, 1.508, 1.791 nm for Ca- smectite montmorillonite, respectively (Zhang et al., 2016).

4. UNRAVELING THE DISAGREEMENT REGARDING THE INTERCALATION OF AFB_1 INTO SMECTITE INTERLAYER

Several studies asserted that the main interaction between AfB_1 and smectite occurred at the dicarbonyl groups of AfB_1 with the cations of smectite. Nevertheless, the specific cationic site of smectite involved in this interaction remains a subject of debate, including the possibility of AfB_1 molecule intercalation into the interlayer. Desheng et al. (2005) conducted adsorption tests on Ca-montmorillonite using eight different concentrations of AfB_1 at pH 2 and 9, reporting the amount of AfB_1 adsorbed were 80% and 90%, respectively. Interestingly, no expansion of d_{001}-value was observed before and after the adsorption test, which remained consistently at 1.41 nm. Consequently, Desheng et al. (2005) concluded that the AfB_1 molecule only adhered to the edge of the montmorillonite layer. Aisawa et al. (2018) utilized four raw and one acid treated montmorillonite for adsorption tests conducted at various pH levels ranging from 3 to 9. All raw montmorillonite samples showed high percentages of AfB_1 removal, approximately 90 to 95%, with pH and temperature simply did not affect the adsorption performance. Notably, significant changes in d_{001}-value were also not observed after the AfB_1 adsorption test. Aisawa et al. (2018) concluded that the unchanged d_{001}-value after the adsorption test was attributed to the carbonyl group of AfB_1 adhering to the outermost central cation of the octahedral sheet and the outermost exchangeable cation (Aisawa et al., 2018).

Other findings suggesting that the smectite interlayer is accessible to the AfB_1 were put forward. Deng et al. (2010) pointed based on experiment, there was d_{001}-value change between Ca-smectite and AfB_1-saturated Ca-smectite after adsorption test. These two conditions of sample were analyzed by using XRD with variable temperature. The d_{001}-value of Ca-smectite was plummeted from ~1.52 nm to below 1.0 nm at 150°C. Similarly, the d_{001}-value of AfB_1-saturated Ca-smectite was plummeted from ~1.49 to ~1.3 nm at 100°C, gradually decreasing to 1.2 nm at 200°C, and then slightly dropping to 1.04 nm at 500°C (Deng et al., 2010). Conformable results were also observed by Alam et al. (2015), who also conducted XRD analysis with variable temperatures. They found the basal spacing of Ca-smectite decreased from ~1.5 to ~1 nm after heating at 200°C, while the basal spacing of AfB_1-saturated Ca-smectite exhibited a slight decrease from ~1.49 to ~1.39 nm (Alam et al., 2015). The summary of basal spacing changes can be seen in Table 2.

Several researchers reported that the basal spacing of montmorillonite would be dropped to ~0.9 to ~1 nm under dehydrated condition after heating at range 100 to 200°C (Ali-Bik et al., 2022; Barrientos Velazquez & Deng, 2020; McAtee Jr. & Concilio, 1959; Orolínová et al., 2012; Ribeiro dos Santos et al., 2019). In its original condition, smectite typically contains water molecules in the interlayer. Some reports indicated that the basal spacing of Ca-montmorillonite, sourced from nature or industry and analyzed using XRD without any high-temperature treatment, had range in values ~1.38 to ~1.54 nm, with the most dominant values emerging around 1.4 nm (Agustian et al., 2023; Ali-Bik et al., 2022; Baek et al., 2022; Barrientos Velazquez & Deng, 2020; Goo et al., 2023; Karakaya et al., 2011; Mao et al., 2024; Rinaldi et al., 2023; D. Yang et al., 2022). Furthermore, the other researchers, Watanabe and Sato (1988), reported basal spacing values at relative humidity (RH) of 0% and 100% for homoionic Na, K, Ca saturated smectite montmorillonite, respectively, as follows: ~1.00 and ~1.88 nm, ~1.03 and ~1.24 nm, ~1.16 and ~1.88 nm. In addition, Tamura et al. (2000) also reported basal spacing values at RH of 0% and 95% for homoionic Na, Li, K, Ca, Mg saturated smectite, respectively, as follows: ~0.99 and ~1.5 nm, ~1.01 and ~1.51 nm, ~1.0 and ~1.26 nm, ~1.19 and ~1.81 nm, ~1.39 and ~1.85 nm.

Based on the comparison, we determine that the initial condition of samples observed by Desheng et al. (2005) and Aisawa et al. (2018) were in hydrated condition and did not experience an increase in basal spacing after adsorption test; hence it is proposed that the carbonyl group of AfB_1 was attached to the water molecules in the interlayer through hydrogen bonding mechanism, as suggested by Deng and Szczerba (2011) and Alam et al. (2015).

Table 2. Comparation d_{001}-value from XRD analysis before and after AfB_1 adsorption

Ref	**Sample code**	**d_{001}-value (nm)**		**Temperature during XRD analysis (°C)**
		before AfB1 adsorption	after AfB1 adsorption	
Desheng et al. (2005)	Mont.	1.41	1.41	-
Aisawa et al. (2018)	MT-1	1.52	1.54	-
	MT-2	1.52	1.52	-
	MT-3	1.50	1.50	-
	MT-4	1.59	1.57	-
Deng et al. (2010)	SM (before adsorption)/AfB1-Ca-Sm (after adsorption)	~1.52	~1.49	~30
	SM (before adsorption)/AfB1-Ca-Sm (after adsorption)	~0.98	~1.3	150
	SM (before adsorption)/AfB1-Ca-Sm (after adsorption)	~0.98	~1.03	500
Alam et al. (2015)	3MS (before adsorption)/AfB1-3MS-in water (after adsorption)	~1.5	~1.49	~30
	3MS (before adsorption)/AfB1-3MS-in water (after adsorption)	1	~1.39	200

The hydration of smectite is affected by various factors, including the extent and location of layer charge, cation species in the interlayer, the water activity, temperature, external pressure, and salinity of the bulk solution (Villar et al., 2012). Therefore, the length of swelling can vary even among distinct smectite minerals.

5. FUTURE CHALLENGE

In this study, we develop a new hypothesis based on the primary conclusion that if hydrogen bonding plays role when the interlayer of smectite is in hydrated condition, then smectite with higher hydration state and longer swelling length will exhibit higher adsorption capacity. To validate this finding, adsorption test of AfB$_1$ should be performed using smectite samples from single origin to ensure relatively uniform layer charge and its distribution. Samples with single homogeneous cation, saturated with Na, Ca, K, Li, and Mg, respectively, should be prepared, as the type of interlayer cation influences swelling behaviour. Following the adsorption test, variable temperature XRD analysis is performed, similar to the method conducted by Deng et al. (2010), to determine the basal spacing values for both adsorbed and non-adsorbed samples under saturated and dry conditions. Building upon hypotheses adapted from previous study by Tamura et al. (2000), it is expected that K-saturated smectite will experience the least swelling, while Mg-saturated smectite will exhibit the highest swelling. Consequently, it is also presumed that K-saturated smectite will have the lowest AfB$_1$ adsorption capacity, while Mg-saturated smectite will have the highest. Therefore, the more water molecules penetrate the interlayer, the more AfB$_1$ molecules are attracted (Fig. 4).

Another hypothesis is also further developed based on the variable temperature XRD analysis result of Deng et al., which suggested that AfB$_1$ molecules remaining in the interlayer were docked in the interlayer cations in dry conditions (Deng et al., 2010). Therefore, it is assumed that the decrease in basal spacing values of each AfB$_1$ adsorbed smectite in dehydrated condition will follow consistent sequence pattern observed when in hydrated condition, with the lowest for K-saturated smectite and the highest for Mg-saturated smectite.

Another important consideration that must be noted is the determination of the maximum adsorption capacity ($Q_{\max}$) if the hypothesis based on the change in basal spacing values is correct. The crucial factor here is whether the Langmuir adsorption isotherm, typically applied in such cases (Alam & Deng, 2017; D'Ascanio et al., 2024; Daković et al., 2012; Wang et al., 2020), is suitable for this type of adsorption. The Langmuir model is developed based on the assumption of the formation of monolayer by adsorbate molecules covering the entire surface of the adsorbent. Smectite is commonly utilized as an adsorbent to remove cationic contaminant from aqueous solution because of the negative charge on its surface, and the Langmuir model usually employed to find the $Q_{\max}$ (Amutenya et al., 2022; Iskander et al., 2011; Prastistho et al., 2018), however, the adsorption of AfB$_1$ onto smectite has different mechanism.

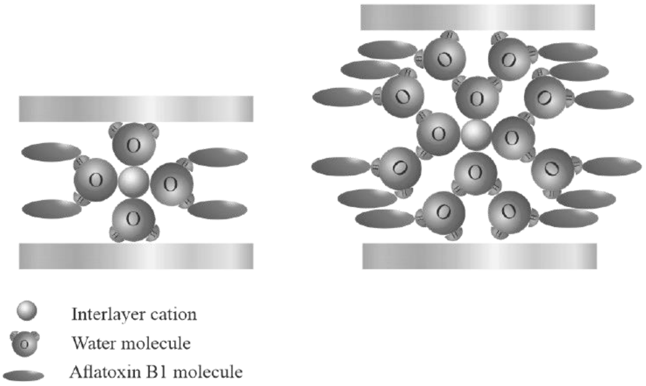

Figure 4. Schematic illustration: greater hydration state of smectite results in higher adsorption capacity of AfB$_1$

6. CONCLUSION

This study clarifies the contradictory interpretations concerning AfB$_1$ intercalation within smectite interlayer in previous studies. The absence of basal spacing expansion after adsorption was attributed to the initial samples condition were not in the expected dry condition (~0.98 to 1 nm), but instead exhibited values ranging 1.4 to 1.59 nm. This finding supports the conclusion proposed in previous research that hydrogen bonding played role when the mineral was in wet condition, while ion-dipole attraction occurred when the mineral in dry condition. However, further researches are required to reinforce this conclusion by elucidating the relationship between hydration state and AfB$_1$ adsorption capacity. A confirmed relationship would necessitate the development of adapted adsorption model.

Declaration of Competing Interest

The authors declare that no competing financial or personal interests may appear to influence the work reported in this paper.

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