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**Nanotechnologies — Performance  
evaluation of nanosuspensions  
containing clay nanoplates for quorum  
quenching**

*Nanotechnologies – Évaluation des performances des  
nanosuspensions de nanofeuillets d'argile pour le quorum quenching*

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## Foreword

ISO (the International Organization for Standardization) is a worldwide federation of national standards bodies (ISO member bodies). The work of preparing International Standards is normally carried out through ISO technical committees. Each member body interested in a subject for which a technical committee has been established has the right to be represented on that committee. International organizations, governmental and non-governmental, in liaison with ISO, also take part in the work. ISO collaborates closely with the International Electrotechnical Commission (IEC) on all matters of electrotechnical standardization.

The procedures used to develop this document and those intended for its further maintenance are described in the ISO/IEC Directives, Part 1. In particular, the different approval criteria needed for the different types of ISO documents should be noted. This document was drafted in accordance with the editorial rules of the ISO/IEC Directives, Part 2 (see [www.iso.org/directives](http://www.iso.org/directives)).

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Any trade name used in this document is information given for the convenience of users and does not constitute an endorsement.

For an explanation of the voluntary nature of standards, the meaning of ISO specific terms and expressions related to conformity assessment, as well as information about ISO's adherence to the World Trade Organization (WTO) principles in the Technical Barriers to Trade (TBT), see [www.iso.org/iso/foreword.html](http://www.iso.org/iso/foreword.html).

This document was prepared by Technical Committee ISO/TC 229, *Nanotechnologies*.

Any feedback or questions on this document should be directed to the user's national standards body. A complete listing of these bodies can be found at [www.iso.org/members.html](http://www.iso.org/members.html).

## Introduction

Among the abundant minerals in the earth's crust, the naturally occurring clays in the class of phyllosilicates such as smectite, talc and mica are layer silicates existed as laminated mass. The stacks of smectite can be exfoliated into individual clay nanoplates of high surface area and high charge density on the surface. Due to the presence of the surface charges, the clay nanoplate gives rise to a strong electrostatic and charge attraction on microbial surface. The clay nanoplates can be further modified by introducing various surfactants to enhance their functions for inhibiting bacterial growth through quorum quenching interactions. The clay nanoplate suspension in water is designed to inhibit the growth of pathogenic bacteria for crop protection from diseases. Moreover, as an additional benefit, harvesting yield increased.

The antibacterial efficacy is attributed to the unique combinations of chemical and physical properties including the nanoplate shape and size dimension, high surface area, ionic charge attraction and water dispersion stability. These combined characteristics in a single nanoplate enable for a long-term antibacterial effect. The inter-relation between clay nanoplate characteristics and antibacterial performance are described in [Annex A](#). The quorum quenching ability depends on the interaction of clay nanoplates with bacterial signaling molecules and bacterial surfaces. It can be used as the standard for quality control for the clay nanoplate, and more importantly, the antimicrobial efficacy by using clay nanoplates can be measured and predicted. The correlation between quorum quenching ability and antibacterial performance is described in [Annex B](#).

This document does not cover safety and environmental aspects. Some safety of clay nanoplate regarding the cytotoxicity and genotoxicity toward human cell, oral lethal dose (LD<sub>50</sub>), and aquatic toxicity are described in [Annex C](#).

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# Nanotechnologies — Performance evaluation of nanosuspensions containing clay nanoplates for quorum quenching

## 1 Scope

This document specifies the performance evaluation of nanosuspensions containing clay nanoplates for quorum quenching in crop production. This document does not cover safety and environmental aspects.

## 2 Normative references

There are no normative references in this document.

## 3 Terms and definitions

For the purposes of this document, the following terms and definitions apply.

ISO and IEC maintain terminology databases for use in standardization at the following addresses:

- ISO Online browsing platform: available at <https://www.iso.org/obp>
- IEC Electropedia: available at <https://www.electropedia.org/>

### 3.1

#### **nanosuspension**

fluid nanodispersion where the dispersed phase is a solid

Note 1 to entry: The use of the term “nanosuspension” carries no implication regarding thermodynamic stability.

[SOURCE: ISO/TS 80004-4:2011, 3.5.1]

### 3.2

#### **clay**

naturally occurring or synthetically manufactured material composed primarily of fine-grained minerals, which is generally plastic at appropriate water contents and will harden when dried or fired

Note 1 to entry: Taken from Reference [1].

Note 2 to entry: Although clay usually contains phyllosilicates, it may contain other materials that impart plasticity and harden when dried or fired. Associated phases in clay may include materials that do not impart plasticity and organic matter. Different disciplines have uniquely defined the size of clay particles, and it is for this reason that “fine grained” is used in the definition rather than a precise value. However, because of these size variations from discipline to discipline, it is important that the particle size be specified in the context of the application.

[SOURCE: ISO/TS 21236-1:2019, 3.4]

### 3.3

#### **nanoplate**

nano-object with one external dimension in the nanoscale and the other two external dimensions significantly larger

[SOURCE: ISO/TS 80004-2:2015, 4.6]

3.4

**clay nanoplate**

nanoplate composed of clay

[SOURCE: ISO/TS 21236-2:2021, 3.3]

3.5

**critical micelle concentration**

concentration of a surfactant above which micelles will form

3.6

**minimum inhibitory concentration**

lowest concentration of the clay nanoplate that completely inhibits visible growth of the initial inoculum after incubation at 35 °C for 18 h

3.7

**minimum bactericidal concentration**

lowest concentration of the clay nanoplate that 99,9 % of the final inoculum is killed after incubation at 35 °C for 24 h

3.8

**nano-object**

discrete piece of material with one, two or three external dimensions in the nanoscale

Note 1 to entry: The second and third external dimensions are orthogonal to the first dimension and to each other.

[SOURCE: ISO/TS 80004-2: 2015, 2.2]

## 4 Abbreviations

AFM	Atomic force microscopy
BET	Brunauer–Emmett–Teller
CEC	Cationic exchange capacity
CMC	Critical micelle concentration
DLS	Dynamic light-scattering
ELS	Electrophoretic light-scattering
FB1	Fumonisin B1
ICP-MS	Inductively coupled plasma mass spectrometry
ICP-OES	Inductively coupled plasma optical emission spectrometry
IEP	Isoelectric point
LD <sub>50</sub>	Oral lethal dose, 50 %
MIC	Minimum inhibitory concentration
MBC	Minimum bactericidal concentration
MMT	Montmorillonite
QQA	Quorum quenching ability

SEM	Scanning electron microscope
TEM	Transmission electron microscopy
XRD	X-ray Diffractometer
XRF	X-ray Fluorescence Spectrometer

## 5 Characteristics and measurement methods

### 5.1 General

The characteristics and measurement methods of clay nanoplate are listed in [Table 1](#). The essential characteristics of clay nanoplate can provide understanding of the potential quorum quenching ability and antibacterial performance. Characteristics can be determined using the methods listed in [Table 1](#). The inter-relation between clay nanoplate characteristics and antibacterial performance are described in [Annex A](#).

**Table 1 — Characteristics of clay nanoplate to be measured**

Characteristics	Units	Measurement method	Test specimen form	Relevant documents
Chemical composition	wt %	ICP-MS or XRF	Powder	ISO/TS 21236-1
Mineral composition	wt %	XRD	Powder	ISO/TS 21236-1
Specific surface area	m <sup>2</sup> /g	BET	Powder	ISO 9277
Cation exchange capacity	meq/100 g	Ammonium acetate method	Powder	ISO 22171 <sup>a</sup>
Hydrodynamic size	µm	DLS	Suspension	ISO 22412
		Laser diffraction method		ISO 13320
Zeta potential	V	ELS	Suspension	ISO 13099-2

<sup>a</sup> Under preparation. Stage at the time of publication: ISO/DIS 22171:2023.

### 5.2 Chemical composition

The chemical composition of clay nanoplate can be determined using ICP-MS, ICP-OES or XRF. The measurement results of the constituent oxides fractions are expressed as wt %.

### 5.3 Mineral composition

The mineral composition of clay nanoplate can be determined using XRD. The XRD pattern could provide the crystalline mineral phase with their corresponding d-spacing values, the measurement results are expressed as wt % to the mineral composition.

### 5.4 Specific surface area

The high surface area with the surface charge exposure in water enables the function of inhibiting microbial growth. The specific surface area of clay nanoplate can be determined according to ISO 9277. This standard specifies the determination of the overall specific external and internal surface area of samples by measuring the amount of physically adsorbed gas according to the BET method.

## 5.5 Cation exchange capacity

The generation of the negative surface charge of clay nanoplate is described by the isomorphous substitution of Si, Al or Mg in clay crystal and balanced by the adsorbed counter ions (cations). The cation exchange capacity indicates how many cations can be exchanged on the surface of silicates expressed as meq/100 g. The ammonium acetate at pH 7 method proposed by Schollenberger et al.<sup>[2]</sup> is widely used to determine the cation exchange capacity. ISO/DIS 22171<sup>1)</sup> specifies a method for the determination of cation exchange capacity and the content of exchangeable cations (Ca, K, Mg, Na) in soils using ammonium acetate solution at pH 7 as extractant.

## 5.6 Hydrodynamic size

Clay nanoplate samples are typically powders. Powder samples shall be well dispersed in the aqueous suspension for size measurement, a higher concentration of clay nanoplate can cause agglomeration. Suspensions often need to be diluted to minimize agglomeration prior to the size measurement. The hydrodynamic diameter of clay nanoplate generally is larger than the primary particle size observed in TEM, SEM or AFM images due to the additional hydration layer, or possible aggregation/agglomeration. The hydrodynamic size shall be measured using DLS or the laser diffraction method according to ISO 22412 or ISO 13320 respectively.

## 5.7 Zeta potential

Zeta potential reflects the surface charge characterization of a particle and its dispersion stability in aqueous suspension; a higher value of zeta potential (absolute value) indicates a high surface charge, leading to strong repulsion forces between charged particles to prevent aggregation. A low value of zeta potential increases the probability of aggregation due to van der Waals attraction.

The differences are lied on the exfoliated clays, that is silicate nanoplates, a surface totally exposed silicates from the pristine clay stacks. With a significantly different charge behaviour from the clay, the clay nanoplates usually exhibit an isoelectric point (IEP) and pH functions of zeta potential. An apparent coagulation can occur when the pH is below the IEP at high edge charge density.<sup>[9]</sup>

The pH value shall be reported along with the zeta potential. ISO 13099-2 specifies a method of measurement of electrophoretic mobility of particles suspended in a liquid for calculating zeta-potential.

## 6 Performance of quorum quenching

### 6.1 Quantification of quorum quenching ability by surfactants

The quorum quenching ability depends on the interaction of clay nanoplates with bacterial signaling molecules and bacterial surfaces. It is the measurement and indication of the non-covalent affinity of the clay nanoplate with polar organic molecules or the microbial cell surface. Three types of commonly used surfactants are selected as the representatives including cationic (dodecyltrimethylammonium bromide), nonionic (octylphenol ethoxylate) and anionic (sodium dodecyl sulfate) surfactants for measuring the quorum quenching ability.

The surface tension with the Wilhelmy plate method is measured by varying the quantity of clay nanoplate to individual surfactant at the original point of the CMC, indicating the quantitative effect on the intrinsic characteristics of the surfactant CMC. The alternation of the surface tension in the titration process of clay nanoplate adding to surfactant is measured. A sharp change in surface tension can be observed at the equivalent point of a titration.<sup>[10]</sup>

In the beginning of the titration process, the small volume of clay nanoplate solution in specified concentration is slowly added to the surfactant solution at the point of CMC. In general, during the measurements of surface tension drops, the time to reach an equilibrium is necessary before the final

1) Under preparation. Stage at the time of publication: ISO/DIS 22171:2023.

data reading. The completion of the reaction is monitored in reaching its equilibrium or no further change of surface tension reading. The added amount of the clay is plotted against the surface tension changes at the surfactant CMC.

The  $QQA$ , expressed in units of meq/100 g, is calculated at the equivalent point with [Formula \(1\)](#):

$$QQA = \frac{(Ws / Es) \cdot 10^5}{Wc} \quad (1)$$

where

$Wc$  is the clay nanoplate concentration at the equivalent point, wt %;

$Ws$  is the surfactant concentration at the equivalent point, wt %;

$Es$  is the surfactant equivalent weight, in g/eq;

$10^5$  is the factor to convert eq/g to meq/100 g.

The change of surface tension at CMC actually represents the quorum quenching ability toward the specific surfactant. The cationic surfactant has a strong ionic exchange or formation of ionic bonds on the clay nanoplate surface and equivalent ratio at critical point. The nonionic surfactant is less affected due to the lacking of ionic exchange interaction but only by C-O bonding dipole-dipole interaction with the added clay nanoplate, it represents a weaker interaction than the charge species. The anionic surfactant has no noticeable interaction on the addition of clay nanoplate due to the absence of both charges and dipole- dipole interactions.

The quantitative strength of interaction between clay nanoplate and surfactant is dependent on the types of the surfactants, in general, the opposite charge interaction is the strongest for the quorum quenching ability or binding affinity.

## 6.2 Antibacterial activities

The MIC and MBC can provide information on antibacterial activities of clay nanoplate potentially being bacteriostatic or bactericidal.

### 6.2.1 Determination of MIC

The MIC of clay nanoplate can be determined with a broth microdilution method according to CLSI M07-11 or ISO 20776-1. The tests are performed in sterile 96-well plastic microdilution trays by making serial twofold dilutions of clay nanoplate, the final test bacterial concentration in each well should be approximately  $5 \times 10^5$  CFU/ml ( $2-8 \times 10^5$  CFU/ml). At least each tray should be included as a growth control well and an uninoculated negative control well. After incubating at  $(35 \pm 2)^\circ\text{C}$  for  $(18 \pm 2)$  h, the MIC is determined by observing the lowest concentration of clay nanoplate that completely inhibits visible growth of the bacterium in the wells. The MIC tests should be performed in triplicate.

### 6.2.2 Determination of MBC

The MBC of clay nanoplate can be determined with a broth microdilution method according to CLSI M26-A. In general, the MBC is determined by first performing the microdilution test procedures for MIC, then, sub-culturing samples from the wells of MIC assay having no visible growth onto the agar plates and incubating the plates for 24 h. The MBC is measured as the lowest concentration of the clay nanoplate that 99,9 % of the final inoculum is killed.

The antimicrobial activity is nonspecific for a diverse range of bacterial species in relation to the physical capturing mechanism enabled by the clay nanoplate.<sup>[11]</sup>

## 7 Test report

The test report shall contain the following information:

- a) a reference to this document, i.e. ISO/TS 4971:2023;
- b) test results of the clay nanoplate characteristics in [Clause 5](#), [Table 1](#) and the test methods used;
- c) the measurement results of the quorum quenching ability by surfactants including cationic, nonionic and anionic surfactants in [6.1](#);
- d) the measurement results of the MIC and MBC in [6.2](#), at least one gram-positive and one gram-negative bacterial species are tested;
- e) details of any operations not specified in this document or regarded as essential.

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## Annex A (informative)

### Relationship between clay nanoplate characteristics and antibacterial performance

#### A.1 General

Naturally abundant clays, such as montmorillonite, halloysite and kaoline, generally have a lamellar structure that comprise multi inorganic plates and counter ions.<sup>[12]</sup> After an exfoliation, the favourable change in geometric shape into thin plates and number of surface charges are found to have a high efficacy for inhibiting bacterial activity. In general, the efficacy is attributed to their high specific surface area, surface charges and fine dispersion in aqueous suspension, which render the attraction forces for any polar organics including bacterial surface.

#### A.2 Particle size and specific surface area

The particle size is dependent on the structure type of clay mineral, it is estimated to be 80 nm to 400 nm for montmorillonite and 300 nm to 1 000 nm for mica. The clay with different lateral dimension may affect the spacing enlargement and surface exposure.<sup>[13]</sup> For example, the specific surface area of montmorillonite, exfoliated montmorillonite, mica and exfoliated mica are about 750 m<sup>2</sup>/g, 800 m<sup>2</sup>/g, 9 m<sup>2</sup>/g and 430 m<sup>2</sup>/g, respectively. In general, the exfoliated clays show a higher specific surface area than those before exfoliation. The high surface exposure will increase the contact area with the bacterial cell wall and strengthen its antibacterial activity.<sup>[11]</sup>

#### A.3 Cation exchange capacity

The cation exchange capacity varies greatly depending on the clay sample. The presence of surface charges on clay nanoplate tend to attract polar organics and the microbial cell surface. The physical adhering onto bacterial surface eventually causes cell rupturing, shrinking and then aggregating into lumps. The antibacterial effect of the clay nanoplate exhibits nonspecific for a broad range of gram- negative and gram-positive bacterial species, but affecting most for the species with the high proliferation rate as the ultimate results by the physical capturing and quorum quenching properties.<sup>[11]</sup>

#### A.4 Hydrodynamic size

The hydrodynamic size refers to the total size of core and the surface modified molecules or solvation attached to the core particles. It is an indication of the dispersion stability and aggregation behaviour of clay nanoplates in aqueous suspension. Several factors affect the hydrodynamic diameter, the higher aqueous suspension concentration can lead an aggregated or agglomerate form, or the surface ionic charges can be solvated by the polar organics and water molecules.<sup>[9]</sup> The aggregation and solvation can affect the covering effect of the clay nanoplates and therefore their antibacterial effect.

#### A.5 Zeta potential

The natural clays such as montmorillonite have negative zeta potential, and slight changes at all pH values, if montmorillonite is exfoliated to a clay nanoplate, which exhibits an isoelectric point and an obvious property of pH dependency. The phenomenon is mainly a result of the clay nanoplate containing large number of newly exposed hydroxyl functionalities on the surface, which originally existed

before exfoliation within the layered structure. We can modify the surface charge of clay nanoplate by introducing various surfactants to change the zeta potential and the charge type.<sup>[14]</sup>

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## Annex B (informative)

### Correlation between quorum quenching ability and antibacterial performance

The clay nanoplate has been shown to degrade the quorum sensing signal molecules into small fragments and disrupt bacterial activity in various media,<sup>[15]</sup> this is another observation that clay materials provide the ability to quench quorum sensing signal molecules. The quorum quenching ability (QQA) can be quantified and expressed in units of meq/100 g. Molecularly well-defined surfactants representing each class of cationic, nonionic and anionic species are selected and used as the standard for measuring their nanoplate interaction. Since each surfactant has its own characteristic CMC value, the QQA is measured and probed by the interaction of clay nanoplates with surfactants. In other words, the original surfactant CMC can disappear due to the nanoplate interaction. The noncovalent bonding between clay and surfactant cause three different CMC-breaking values, for examples, strong ionic attraction for cationic surfactant, relatively weak dipole-dipole interaction for nonionic surfactant, and interaction with edge hydroxyl groups for anionic surfactant.

Three different classes of surfactants are employed for simulating the actions of quorum sensing molecules emitted by bacterial colonies. The QQA becomes an important parameter for estimating the effectiveness of nanoplate interacting with the bacterial communication molecules of different species. The value of QQA by calculating [Formula \(1\)](#) to the cationic surfactant HDTMA ( $C_{16}H_{33}N(CH_3)_3Br$ ) and DDTMA ( $C_{12}H_{25}N(CH_3)_3Br$ ) are 110 meq/100 g and 108 meq/100 g respectively.<sup>[10]</sup> This value nears the number of surface charge per clay nanoplate (approximately 120 meq/100 g),<sup>[11]</sup> indicating the capacity of ionic charges enabling an ionic exchange reaction in an equivalent manner for the negatively charged clay nanoplate when quenching cationic surfactant. In contrast, the non-ionic surfactant, octylphenol ethoxylate ( $C_{14}H_{22}O(C_2H_4O)_n$  ( $n = 9-10$ )), the value of QQA is 0,512,<sup>[10]</sup> by the force of dipole-dipole interaction. And there is none of significant interaction by observing the change toward the surfactant's CMC. Hence, depending on the chemical nature of bacterial quorum sensing signals, the nanoplates tend to express a different quorum quenching ability that subsequently affecting the bacterial proliferation. Hence, the quenching ability can be the measurement for the nanoplate's quality and the subsequent efficacy for inhibiting bacterial growth.

Further, after nanoplate quenching the quorum sensing signal, it continues to adhere onto bacterial surface and can subsequently block the signal emission in a similar manner of noncovalent bonding affinity toward the surface of bacterial cell walls. The QQA, particularly the ability measured by the one with the positively charged cationic surfactant, is applicable for blocking bacterial surface for presumably inhibiting the quorum sensing. Therefore, the overall antimicrobial efficacy is correlated with the nanoplate's noncovalent bonding force but depending on the surface characteristics of the microorganisms. The bacterial cell walls have a net negative surface charge<sup>[16]</sup> and consist of variety of chemical moieties including carboxyl, amide, phosphate, hydroxyl and carbohydrate related moieties,<sup>[17]</sup> implying the strong affinity for nanoplate. Based on the experimental data, the MBC value to negatively charged clay nanoplate is 3 000 ppm,<sup>[11]</sup> as this clay nanoplate is modified by the cationic n-octadecanyleamine cation ( $n-C_{18}H_{37}NH_3^+$ ), the MBC value is dropped by 10 ppm.<sup>[14]</sup> There is a 300-fold difference in the concentration required to inhibit bacterial growth between negatively charged clay nanoplate and modified clay nanoplate. This phenomenon has confirmed the concept of QQA measurement. Antimicrobial efficacy by using nanoplates can be measured and predicted, and more importantly, can be used as the standard for quality control for the clay nanoplate products. Overall, the QQA is pragmatically used to deal with the pathogenic bacteria and a broad spectrum of the bacterial colonies in agriculture fields and soils.

## Annex C (informative)

### Safety of clay nanoplates

#### C.1 Natural clay

Bentonite clay has been on the FDA's GRAS list (Generally Recognized as Safe) for many years (CFR-Code of Federal Regulations Title 21 - 21CFR184.1155).<sup>[18]</sup> Bentonite, a colloidal clay (CAS Registry Number<sup>2)</sup> 1302-78-9) consists primarily of montmorillonite (MMT). Bentonite contains varying quantities of iron, alkalis and alkaline earths in the commercial products.

#### C.2 Clay nanoplate

The cytotoxicity and genotoxicity of the clay nanoplate derived from the exfoliated MMT was studied by Li et al.<sup>[19]</sup> The author found that the Comet assay test on Chinese Hamster Ovary (CHO) cells showed no DNA damage after 24 h of incubation with clay nanoplate of 1 000 µg/ml, indicating none of genotoxicity. The micronucleus test indicated no significant chromosomal damage on mice for doses up to 500 mg/kg body weight and the Salmonella gene mutation assay on strains TA98, TA100, TA102, TA1535, and TA1537 also showed no effect on the mutagenicity. Furthermore, cytotoxicity of the clay nanoplate was assayed by 3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide and lactate dehydrogenase release from MTT and layered double hydroxides, respectively, showing low cytotoxicity on CHO cells below 1 000 µg/ml after 12 h incubation period and a dose-dependent effect after 24 h incubation. For feeding to rats, the acute oral toxicity was very low or similar to the toxicity of NaCl. Many toxicological evaluation results have been reported for commercially available clay nanoplate products in a scientific literature. Maisanaba et al.<sup>[20]</sup> observed the variety of the toxicity conclusions described in the scientific literature, the author found that unmodified clay nanoplates in general induced cytotoxicity dependent on the clay concentration, exposure time and experimental system. Most quaternary ammonium modified clay showed clear evidences of toxicity.

Most of the previous studies in rodents treated with unmodified MMT showed no clear evidence of toxicity.<sup>[20-21]</sup> Li et al. evaluated the LD<sub>50</sub> of the clay nanoplate in rats, the LD<sub>50</sub> was larger than 5,700 mg/kg by body weight of female Sprague-Dawley rats.<sup>[19]</sup> Afriyie-gyawu reported that long-term dietary exposure to MMT at 2,0 % in Sprague-Dawley rats for 28 w did not show the potential toxicity.<sup>[22]</sup> Liao et al. observed that clay nanoplate showed high binding capacity for mycotoxins such as for FB1 and did not disturb the mouse embryos development, the newborn mice from females consumed with clay nanoplate showed no abnormalities.<sup>[23]</sup> Abdel-Wahhab et al. reported that MMT was added to the diet at a level of 0,5 % and fed to the pregnant rat throughout pregnancy, the MMT alone was not toxic and can tightly bind aflatoxin in the gastrointestinal tract of animals.<sup>[24]</sup> A modified with a cetyltrimethylammonium bromide MMT for different biochemical and histological analyses was investigated by El-Nekeety et al.<sup>[25]</sup> The author found that modified MMT alone was safe and even beneficial to reduce the toxicity of FB1 and zearlenone in Sprague-Dawley rats.

#### C.3 Effect of clay nanoplate on aqueous organisms

Drinking water safety has been threatened by increasing harmful algal blooms in water sources. At the dosage of 100 ppm, the clay nanoplate can enhance the settling of suspended *Microcystis aeruginosa*, and adsorb more than 99 % of microcystin-LR at a dosage of 500 ppm.<sup>[26]</sup> Aqueous organisms are widely used in toxicity tests performed with hazardous environmental contaminants released from water or

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