Synthetic Reactive Dye Wastewater Treatment by Narrow-leaved Cattail: studied by XRD and FTIR

S. Nilratnisakorn¹, P. Thiravetyan²,*, and W. Nakbanpote³,#

¹The Joint Graduate School of Energy and Environment,
²School of Bioresources and Technology,
³Pilot Plant Development and Training Institute,
King Mongkut’s University of Technology Thonburi,
Bangkok 10150, Thailand.

* Corresponding author: Tel: + 662-4707535, Fax: + 662-4523455
E-mail address: paitip.thi@kmutt.ac.th

# Present address: Department of Biology, Faculty of Science,
Mahasarakarm University, Mahasarakam 44150, Thailand.

Abstract: Narrow-leaved cattail (Typha angustifolia Linn) has been reported as being useful in the removal of textile dyes from industrial sources. This study investigated the possible mechanism for plant avoidance in this wastewater by X-ray diffraction (XRD) and Fourier transform infrared spectroscopy (FTIR). Evidence from XRD showed the precipitation of dye with silicon (Si) and calcium (Ca) oxalate in the plant tissue. FTIR spectra indicated that amide (R(C=O)NH₂) and siloxane (Si-O-Si) groups in the plant might be involved in the dye
removal. This implied that the decolorization of textile wastewater by narrow-leaved cattails involved an amide group or protein and silicon such as a complex of iron-calcium-silicate to bind with the negative charge of dye and/or wastewater. After uptake and translocation in the plant system, the semi-permeability system of plant might select and avoid the solute as dye molecule by several mechanisms such as precipitation by iron-calcium-silicate complexes or dye degradation. High intensity XRD peaks from calcium oxalate (CaC$_2$O$_4$), calcium silicate (Ca$_2$SiO$_4$) complexes and silica (SiO$_x$) were also observed in these samples suggesting that some metals might be involved in SRDW removal by this plant.

**Keywords:** Narrow-leaved Cattail, reactive dye, FTIR, XRD.

### 1. Introduction

In 2007, the export quantities of Thailand’s garment and textile sectors were approximately 261 Billion Baht [1]. This corresponded to the release of $1.2 \times 10^{11}$ liters of effluents from the textile industry into public streams [1]. The characteristics of textile wastewater are high pH, alkalinity and the presence of various soluble dye compounds which is highly visible, and it is very difficult to degrade or eliminate [2]. Decolorization of textile wastewater can be accomplished through many efficient methods, including physical, chemical and biological treatment. Treatment with algae, fungi and bacteria are biological methods that have been reported for the biodegradation of reactive azo dye [3-9].
Phytoremediation for treatment of textile wastewater is an alternative method and is sustainable for long-term treatment. It is a low cost technique, with low chemical and energy consumption. It is easy to operate and to maintain the treatment system using this method [10]. Decolorisations of azo dyes has been investigated in wetlands of Phragmites [8,11], and Saccharum [12]. Until recently there were no reports of the use of narrow-leaved cattails (Typha angustifolia Linn.) for textile dye removal from wastewater. Through our preliminary study, we found that this plant can efficiently remove dye and treat textile wastewater by decreasing pH, COD and TDS [13]. Therefore, the objectives of this study were to (a) identify the functional groups in the plant that might be involved in textile dye removal and (b) determine the possible mechanism for textile dye wastewater treatment by narrow-leaved cattail.

2. Materials and methods

2.1 Dyes and Synthetic Reactive Dye Wastewater (SRDW)

Synthetic Reactive Dye Wastewater (SRDW) was prepared in a laboratory dyeing process and contained 400 mg l^{-1} of RR141, with 90 g l^{-1} of sodium sulphate and 20 g l^{-1} sodium carbonate being added to increase the dye substance and to improve the fastness in the dyeing process. The commercial diazo C.I. Reactive Dye (Reactive Red 141: Molecular Structure = \text{C}_{52}\text{H}_{26}\text{Cl}_{2}\text{N}_{14}\text{Na}_{8}\text{O}_{26}\text{S}_{8}) used in this study was obtained from DyStar, Thai Co.,Ltd. Thailand. The final pH of the SRDW was approximately 10-11. The maximum absorbance of SRDW was at \lambda_{\text{max}} = 544 \text{ nm} (determined by UV-visible
spectrophotometer, model UNICO-2100, USA). In this study, the SRDW was diluted from 400 mg/l to 20 mg/l, which is similar to the concentration of dye residues from textile effluents in public waterways [14], and the initial pH was adjusted to 9.0.

2.2 Plant culture conditions

Narrow-leaved cattails (Typha angustifolia Linn.) were collected from a freshwater pond near King Mongkut’s University of Technology Thonburi (KMUTT) Bangkhuntien Campus, Thailand, and maintained in plastic boxes until new shoots were produced. Plants were selected and cultured in fresh pond water containing added SRDW as described in section 2.1. Plants were selected at the same stage of growth (4-5 leaves per plant, 20-30 roots per plant, 90-100 cm. height,) for growing in 10” width x 15” length of glass bottles with and without added clay. The volumes of solution in each treatment were adjusted to 1500 ml. per bottle. Plants were cultured for 28 days, samples analyzed at days 0, 7, 14, 21, and 28.

2.3 Functional groups analysis and mechanism of textile wastewater treatment by narrow-leaved cattail.

2.3.1 X-ray Diffraction (XRD) Study

XRD was used to measure the crystalline dye and the other chemical compounds in plants after treatment with SRDW. Samples were analyzed by X-ray diffractometer (JEOL, JDX-3530) using a 30 kV voltage and 40 mA current. The diffraction angel of 5- 100°2θ were scanned in steps of 0.02 degree per second.
2.3.2 Fourier Transmission Infrared (FTIR) Spectrophotometer Study

Narrow-leaved cattail before and after treatment with 20 mg/l SRDW were air dried using solar energy. Plants were then ground with an agate pestle and then analyzed by FTIR (PerkinElmer Spectrum One) to determine the functional groups involved in the dye degradation mechanism. The spectra were obtained using the KBr disc technique with a ratio of 1 mg of sample per 100 mg of KBr.

3. Results

3.1 Effect of calcium oxalate (CaC₂O₄), calcium silicate (Ca₂SiO₄) and silicon (SiOₓ) for enhancing SRDW treatment of narrow-leaved cattail by XRD

XRD results revealed that the polyaniline structure of reactive red 141 and the sodium salts (Na₂SO₄ and Na₂CO₃), which were added to the SRDW, were present in the leaves and roots of narrow-leaved cattails after treatment with SRDW (Figures 1-2). High intensity XRD peaks from calcium oxalate (CaC₂O₄), calcium silicate (Ca₂SiO₄) complexes and silica (SiOₓ) were also observed in these samples suggesting that some metals might be involved in SRDW removal by this plant. Therefore, this plant might deal with the toxic dye by cutting it into smaller molecules, which could be easily translocated to areas (e.g. vacuole, golgi body, vesicle and etc.) by semi-permeable membrane of plant, that do not interrupt photosynthesis and solute transport.
Figure 1. X-ray Diffraction patterns of leaf before and after SRDW treatment (A) SRDW showing the dye structure with polyaniline and Na$_2$SO$_4$ + Na$_2$CO$_3$ (Bur.=Burkeite) (B) leaf of narrow-leaved cattail as a control plant showing the pattern from the C-6 backbone (D-glucose, D-galactose) and (C) leaf of narrow-leaved cattail after 28 days exposure to SRDW showing the pattern from the dye, Na$_2$SO$_4$ + Na$_2$CO$_3$, calcium (mono-, di-) oxalate, calcium silicate and silica. At $2\theta \sim 45^\circ$ peaks from dye and silica overlap.
Figure 2. X-ray Diffraction patterns of root before and after SRDW treatment (A) SRDW showing the dye structure with polyaniline and Na\textsubscript{2}SO\textsubscript{4} + Na\textsubscript{2}CO\textsubscript{3} (Bur.=Burkeite) (B) root of narrow-leaved cattail as a control plant showing the pattern from the C-6 backbone (D-glucose, D-galactose) and (C) root of narrow-leaved cattail after 28 days exposure to SRDW showing the pattern from the dye, Na\textsubscript{2}SO\textsubscript{4} + Na\textsubscript{2}CO\textsubscript{3}, calcium (mono-, di-) oxalate, calcium silicate and silica. At $2\theta \sim 45^\circ$ peaks from dye and silica overlap.
3.2 Effect of SRDW treatment of narrow-leaved cattail on FTIR of amide and siloxane groups

FTIR spectra of narrow-leaved cattail were investigated after 28 days of exposure to SRDW. The spectrum of control set revealed that SRDW consisted of peaks from primary and secondary amines (around 3500-3200; NH stretching and 1568 cm$^{-1}$; O=C-NH bending), aromatic azo bond (2112.5 cm$^{-1}$), sulfonate (1117.6; SO$_3^-$ and 900 cm$^{-1}$; R-SO$_3^-$Na$^+$) and chloride (600 cm$^{-1}$). The spectra of plant leaves and roots, before and after SRDW treatment, indicate that the primary (I) and secondary (II) amide groups (3314.5, 2932.2, 1738.4, 1642.9, 1515.6, 1436 and 1332.5 cm$^{-1}$) and the siloxane group (1038 cm$^{-1}$) of the plant leaf were affected by the SRDW. The band from amide I and II were shifted and the band from the siloxane group shifted and decreased. The aromatic ring (2112.5 cm$^{-1}$) and sulfonate group (1117.6 cm$^{-1}$) of the azo compound replaced the amide II (NH-bending) and C-OH bending of plant cellulose, respectively (Tables 1 – 2).
### Table 1. Peak positions and assignments of FTIR spectra from narrow-leaved cattail leaf (control and SRDW plants).

<table>
<thead>
<tr>
<th>Characteristics</th>
<th>Wavenumber (cm(^{-1}))</th>
<th>Wavenumber (cm(^{-1}))</th>
<th>Absorbance change</th>
<th>Remarks</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Control leaf</td>
<td>SRDW leaf</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Peak Shift</td>
<td>3314.5</td>
<td>3401.8</td>
<td>+77.6</td>
<td>↓ 0.15</td>
</tr>
<tr>
<td></td>
<td>2932.2</td>
<td>2940.2</td>
<td>+8.0</td>
<td>↓ 0.15</td>
</tr>
<tr>
<td></td>
<td>1738.4</td>
<td>2112.5</td>
<td>+324.1</td>
<td>↑ 0.05</td>
</tr>
<tr>
<td></td>
<td>1515.6</td>
<td>1467.8</td>
<td>-47.8</td>
<td>↓ 0.35</td>
</tr>
<tr>
<td></td>
<td>1436</td>
<td>-</td>
<td>-</td>
<td>40.1</td>
</tr>
<tr>
<td></td>
<td>1332.5</td>
<td>-</td>
<td>-</td>
<td>40.3</td>
</tr>
<tr>
<td></td>
<td>1245</td>
<td>1117.6</td>
<td>-127.4</td>
<td>↑ 0.4</td>
</tr>
<tr>
<td></td>
<td>1046</td>
<td>1038</td>
<td>-8.0</td>
<td>↓ 0.35</td>
</tr>
<tr>
<td></td>
<td>-</td>
<td>624.2</td>
<td>-</td>
<td>↑ 0.3</td>
</tr>
<tr>
<td>Absorbance Change</td>
<td>1642.9</td>
<td>1642.9</td>
<td>-</td>
<td>↓ 0.2</td>
</tr>
</tbody>
</table>
Table 2. Peak positions and assignments of FTIR spectra from narrow-leaved cattail root (control and SRDW plants).

<table>
<thead>
<tr>
<th>Characteristics</th>
<th>Wavenumber (cm⁻¹)</th>
<th>Wavenumber (cm⁻¹) change</th>
<th>Absorbance change</th>
<th>Remarks</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Control leaf</td>
<td>SRDW leaf</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Peak Shift</td>
<td>3704.2</td>
<td>3688.3</td>
<td>-15.9</td>
<td>-</td>
</tr>
<tr>
<td></td>
<td>3632.6</td>
<td>3624.6</td>
<td>-8.0</td>
<td>-</td>
</tr>
<tr>
<td></td>
<td>3338.1</td>
<td>3417.7</td>
<td>+79.6</td>
<td>-</td>
</tr>
<tr>
<td></td>
<td>2924.2</td>
<td>2932.2</td>
<td>+8.0</td>
<td>-</td>
</tr>
<tr>
<td></td>
<td>1873.7</td>
<td>1642.9</td>
<td>-15.9</td>
<td>↑ 0.2</td>
</tr>
<tr>
<td></td>
<td>1658.8</td>
<td>1038.0</td>
<td>-8.0</td>
<td>↓ 0.35</td>
</tr>
<tr>
<td>Absorbance Change</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td></td>
</tr>
<tr>
<td>Remarks</td>
<td>amide I, II NH-stretching</td>
<td>amide I, II NH-stretching</td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td>CH asymmetric and symmetric stretching</td>
<td>CH asymmetric and symmetric stretching</td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td>RR=C=CH₂ of dye</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td>O=C-NH bending of amide II in plant</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td>Si-O-Si open chain in plant</td>
<td></td>
<td></td>
<td></td>
</tr>
</tbody>
</table>
4. Discussion

4.1 Effect of calcium oxalate (CaC$_2$O$_4$), calcium silicate (CaSiO$_4$) and silica (SiO$_x$) on enhancing SRDW treatment by narrow-leaved cattail by XRD

Peaks from calcium oxalate (CaC$_2$O$_4$), calcium silicate (Ca$_x$Si$_y$O$_z$) and silica (SiO$_x$) were found in narrow-leaved cattail after culturing in SRDW. This suggested that silica, calcium mono, di-oxalate and calcium silicate might enhance the tolerance mechanism of the plant by forming metal-\textit{dye} complexes which are precipitated in leaf and root cells. Because the dye molecule can act as a barrier to the photosynthesis system, and hence translocation and transportation of nutrient in the plant system, precipitation of the dye compound with CaC$_2$O$_4$, Ca$_2$SiO$_4$ and SiO$_x$ are a good mechanism for achieving tolerance. Observation of Si, Ca, S and Fe in leaf and root cells of narrow-leaved cattail by TEM-EDX [13] support this explanation based on XRD results. Therefore, it should be noted here that the principal roles for Si and Ca are to facilitate precipitation of the dye to avoid damages to plant cells. Calcium competes with sodium from salt stress and leads defenses to salt stress as with SRDW by releasing Si into cell walls and/or membranes. Silicon in cell walls in the form of polymeric silica will be converted to silica gel, which will act as a buffer and enable the plant to adjust the optimum osmotic pressure in the stem and reduce the toxicity from Na. At high Na levels, plants will produce CaC$_2$O$_4$, Ca$_2$SiO$_4$ in order to reduce ion-toxicity and to precipitate crystalline forms and suspensions in cells [15-19]. Calcium ions form Ca$^{2+}$ links in cell
walls. However, in plant cells Ca mainly functions in the signal transduction pathways which involve large numbers of different proteins. External factors that affect plants (e.g. light, temperature, wind, CO₂, pathogen, heavy metal, drought, salt stress) are sensed by Ca²⁺ in cell walls which turns on the signal transduction to induce protein kinase activity. The Ca²⁺ in signal transduction is involved with the proton release in cells, and is correlated with siloxane bond formation by enhancing the activity of homologous enzymes that catalase the formation of siloxane bonds [20]. The proton generate by Ca²⁺ might help plant for relieve the toxic of dye, this proton might play the major role for dye degradation to the small molecule and deposit in plant leaves and roots. Plant of this type might use the phytochemical by releasing the smaller molecules into soil to control pH, and the gaseous composition of the soil, and leading to the altered toxic compound being fixing in the soil. Supporting results relate to the system pH of SRDW-treated plants. During the first week of exposure, the system pH decreased from 9 to close to 7, but during the second week it was close to 8. During this time the plant optimized the systematic pH for biochemical processing of the foreign molecule.

Azo dye degradation or decomposition has been reported in bioremediation by various plants and microorganisms (Table 3). The dye structures with several different numbers of carbon atoms were determined by matching the XRD peaks with the library program. Degradation of the reactive di-azo dye can be explained by 2 possible mechanisms. First, the reactive C-52 atom di-azo dye might
break at the linkage group (Figure 3) to produce one azo dye with C-16 and another with C-29 atoms that still retain the linkage group. This mechanism can be understood in terms of the di-azo dye synthesis, in which two azo dye molecules with C-16 atoms are combined initially. Then one side of the linkage group is modified to enable attachment of another azo dye molecule [21-23].

Table 3. The azo dye degradation or decomposition by bioremediation processes [8,11-12,24-25].

<table>
<thead>
<tr>
<th>Dye (Azo)</th>
<th>Degradation (%)</th>
<th>Methods</th>
<th>References</th>
</tr>
</thead>
<tbody>
<tr>
<td>Acid Orange 7</td>
<td>N/A*</td>
<td>Ascorbate-glutathione pathway by Phragmites australis</td>
<td>Carias et al., 2008</td>
</tr>
<tr>
<td>Methyl Orange</td>
<td>N/A*</td>
<td>Lactobacillus casei TISR1500</td>
<td>Seesuriyachan et al., 2007</td>
</tr>
<tr>
<td>Reactive Red 120</td>
<td>N/A*</td>
<td>Ozonation</td>
<td>Zhang et al., 2007</td>
</tr>
<tr>
<td>Acid Orange 7</td>
<td>100% in 120 h</td>
<td>Peroxidase of P. australis</td>
<td>Davies et al., 2007</td>
</tr>
<tr>
<td>Procion Green (HE-4BD)</td>
<td>100% in 1 h</td>
<td>Peroxidase of Saccharum spontaneum</td>
<td>Shaffqui et al., 2002</td>
</tr>
<tr>
<td>Supranol Green</td>
<td>100% in 1 h</td>
<td>Peroxidase of S. spontaneum</td>
<td>Shaffqui et al., 2002</td>
</tr>
<tr>
<td>Direct Blue</td>
<td>&gt;70% in 1 h</td>
<td>Peroxidase of S. spontaneum</td>
<td>Shaffqui et al., 2002</td>
</tr>
<tr>
<td>Procion Blue (H-7G)</td>
<td>&gt;70% in 1 h</td>
<td>Peroxidase of S. spontaneum</td>
<td>Shaffqui et al., 2002</td>
</tr>
<tr>
<td>Chrysoidine</td>
<td>&gt;70% in 1 h</td>
<td>Peroxidase of S. spontaneum</td>
<td>Shaffqui et al., 2002</td>
</tr>
</tbody>
</table>

*N/A = Not Available
Figure 3. Possible dye molecular breaking from C-52 molecule to C-20 molecule and linkage group (pathway 1).

For the second mechanism, the C-52 molecule might degrade produce a C-29 molecule by breaking at 1,6-di-aminebenzene ring and modified structure of another O-chloro-1,3,5-trinitrobenzene. This process will produce 2 molecules of C-29 atoms and C-23 atoms. Then C-23 molecule will break O-chloro-1,3,5-trinitrobenzene to be C-20 molecule. Then C-20 molecules will modify one side of naphthalene to be C-16 molecule (Figure 4). In the case of narrow-leaved cattail, the possible degradation of reactive azo dye might reduce C-52 to C-20 atoms. Hence, the modification of dye molecule by breaking at the linkage group of dye from C-52 to C-20 are easier than modified O-chloro-1,3,5-trinitrobenzene in the case of C-29, C-20 and C-16 atoms, respectively.
4.2 Effect of amide and siloxane groups on SRDW treatment of narrow-leaved cattail

FTIR spectra suggest that primary and secondary amide and siloxane (Si-O-Si) groups in the plant play important roles in SRDW tolerance. $\text{O=Cs}^+$ and $\text{NH}^+$ of amide I, II and siloxane groups or Si-O-Si bridges can bind with the negatively charged dye compound ($\text{dye}^-$) [26]. The aromatic ring with azo bond (2112.5 cm$^{-1}$) and sulfonate group ($\text{SO}_3^-$) at 1117.6 cm$^{-1}$ which replaced amide II ($\text{NH}$-bending), C-OH or C-O-C bending of cellulose and the increase in $\text{Cl}^-$ peak (624 cm$^{-1}$) indicate that this plant has a mechanism for SRDW translocation and transportation [27]. Evidence for dye movement in the plant stem could be seen as patches along the length of vein in the vascular bundle within 3 hr of exposure. These then reduced progressively and were not observed after 3 days. This suggests that after culturing narrow-leaved cattail in SRDW, semi-permeable membrane properties of plant and tolerance mechanisms function by detecting and selecting ions or molecules that are less toxic to the cells [28-29]. Functional groups which play crucial roles might be amide I, II and siloxane.
Figure 4. Possible dye molecular breaking from C-52 molecule to C-29, C-20 and C-16 molecule (pathway 2).

In salt stress conditions, as with SRDW under alkaline conditions, plants have levels for avoidance, tolerance and finally resistance to this kind of stress. Increasing the number of silica groups (Si-O-Si or SiO$_2$) might help the morphological and chemical changes which responded to the salt stress condition [15,30]. In monocotyledon plant, Si mainly in the cell wall helps maintain the integrity, stability, and function of the plasma membrane, and mitigate salinity toxicity by decreasing the Na$^+$ concentration in shoots. The consequent increased H$^+$ in leaves from salt stress and then Si maintains the optimal membrane fluidity [31-33]. Dye and some metal precipitation (e.g. Si), in the leaf and root cell of narrow-
leaved cattail was also seen in studies using TEM-EDX [13]. The amide I, II or proteins are discussed in detail in section 4.3.

The FTIR pattern for the polysaccharide skeleton (C-O-C) of the plant might represent common mechanism with the cotton dyeing process by attaching the carboxylic group, as occurs in the case of dye deposited in old leaves [8,34].

4.3 The possible mechanism for textile wastewater removal by Narrow-leaved Cattail

The mechanism for textile wastewater by narrow-leaved cattail involves both external and internal mechanisms. The external mechanism found that siloxane (Si-O-Si) was involved in dye absorption and precipitation of the sodium salt in the outer membrane of the plant. For the internal mechanism, FTIR and TEM-EDX showed that Si, Ca and Fe were involved in dye absorption indicating that silica production was induced by SRDW. FTIR showed that the amide groups (NH) have changed, implying that SRDW removal by this plant needs NH from amide groups.

Si, Ca and protein might have functions that are related to each other. At the beginning of the stress condition from SRDW, the alkaline conditions will induce protein kinase and other proteins activity. Protein will then increase from accumulated free proline in the stem, and will help to maintain the moisture and fluidity of the plant and to avoid the toxicity from osmotic stress that results from the salt stress (data not shown). Silica also functions to mitigate salinity toxicity by decreasing the Na$^+$ concentration in shoots of monocotyledon.
plants [31-33,35-37]. Ca might help plants by functioning as a signal transducer, which involves proton released in the cell and acts involves many different proteins. The formation of siloxane bonds also requires Ca to achieve the maximum activity [20].

5. Conclusions

Narrow-leaved cattail shown its effective in dye removal from textile wastewaters and functional groups analyses have found that siloxane (Si-O-Si) and amide (NH) groups in the plant played major roles. XRD showed precipitation of silica (SiO₃), calcium-silicate (Ca₂SiO₄) and calcium oxalate (CaC₂O₄) in the plant tissues. These would be possible that plant could survive in the stress condition of this wastewater which contained of dye and salt by several mechanisms such as the external and internal mechanism. The precipitation with calcium complex or the semi-permeability by silicon might be the avoidance process via external mechanism. The internal mechanism, plant might have the proton or enzyme generation for degradation of reactive azo dye might reduce to C-29 and C-16 by breaking at the linkage group of dye. FTIR spectra of plants showed an increase in the peak from sulfur groups, along with decreases in amide (R(C=O)NH₂) and siloxane (Si-O-Si) groups in plants treated with SRDW. Protein or amide groups might therefore, be involved in the mechanism for textile wastewater treatment by this plant. FTIR and XRD results suggest that silica, calcium-silicate, and calcium oxalate are involved in the precipitation of metals, such as calcium complexes by release silicon and/or calcium from cell
walls and cell membranes. Under the caustic conditions as SRDW, calcium will compete with sodium and Ca\(^{2+}\) will bind with oxalate. Calcium oxalate may bind with negative charge and/or sulfur of dye and produce the crystalline deposit in cell. Protein or amide groups of plant might play a role to bind with dye at NH-group of amide.

**Acknowledgements**

This research is supported by National Research Council of Thailand. Ms. Sumol Nilratnisakorn gratefully acknowledges a Ph.D. Scholarship from the Royal Golden Jubilee Project of the Thailand Research Fund (Grant No.PHD/0246/2546). The authors are grateful Dr. Bernard A. Goodman, Department of Environmental Research, ARC Seibersdorf Resarch GmbH, Austria, for helpful discussions.

**References**


