DOCTORAL THESIS

Investigation of green algae and their application in food and environmental science

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Abstract

Many contaminants, such as industrial chemicals, fertilizers, herbicides, pharmaceuticals and heavy metals are released to e environment. 3,4-dichloroaniline（3,4-DCA） originated from degradation of some herbicides such as diuron, propanil and linuron, is toxic to aquatic organisms and affects human being immune system. Triclosan, widely used as antimicrobial agent in pharmaceuticals and personal care products (PPCPs), has been detected as contaminat in various aquatic environments. In this work, green algae were isolated from local environment, then applied for the removal and biodegradation of 3,4-DCA and triclosan.

Two axenic pure algae were isolated using the solid agar method. One of the algae was identified morphologically as *Desmodesmus* sp. based on the experimental results. The other one was identified morphologically as *Chlorella pyrenoidosa* by accredited authority. At the same time, alga *S. obliqnus* was obtained commercially. All the three green algae were cultured in tris-acetate-phosphate (TAP) medium.

Firstly, the alga *C. pyrenoidosa* was applied to remove and biodegrade 3,4-DCA with a concentration of 4.6 μg/mL for 7 d. A removal percentage of 78.4% was obtained over a 7-d period. Two major metabolites with less toxicity were identified as 3,4-dichloroformanilide and 3,4-dichloroacetanilide using HPLC-ESI-ion trap-MS.

Secondly, all the three green microalgae species including *C. pyrenoidosa*, *Desmodesmus* sp., and *S. obliqnus*, were compared in the removal and biodegradation of triclosan in aqueous medium. When triclosan with concentration of 400 ng/mL was cultured with the three algal species separately, triclosan was quickly eliminated from medium in the 1 d cultivation by algae with removal percentages of 62.4%, 92.9% and 99.7% for *C. pyrenoidosa*, *Desmodesmus* sp. and *S. obliqnus*, respectively. The dominant mechanism for the removal of triclosan by *C. pyrenoidosa* was determined as cellular uptake. Biotransfromation of triclosan involved hydroxylation and methylation, glucose conjugation was determined as the predominant mechanisms for the removal of triclosan by algae *Desmodesmus* sp. and *S. obliqnus*. The intermediates from hydroxylation, reductive dechlorination, or ether bond cleavage were immediately subjected to glucosylation and/or methylation via the hydroxyl group of triclosan or introduced, which served as detoxification mechanisms of the chlorinated aromatic chemicals.

In order to find the intermediates in the metabolic pathway of triclosan by algae, *Desmodesmus* sp. was exposed to 400 ng/mL triclosan. 2,4-DCP was detected during the cultivation period 3-12 h using ultra performance liquid performance (UPLC)-ESI-MS/MS. The metabolites from multi metabolic reaction like the glucose conjugate of hydroxylated triclosan were detected in the first 30 min after exposure. The metabolites as products from glucosylation and consecutive hydroxylation and methylation of triclosan or 2,4-DCP were detected after 3 h

cultivation.

To provide more information about the reductive capability of *C. pyrenoidosa*, the reaction between *C. pyrenoidosa* and triclosan was investigated. When *C. pyrenoidosa* was exposed to triclosan with concentration from 100 to 800 ng/mL, more than 50% of triclosan was eliminated by algal uptake from the culture medium during the first 1 h exposure. In the biodegradation experiments, a major metabolite from the reductive dechlorination of triclosan was identified by using liquid chromatography (LC)-ESI-MS. The ability of reductive dechlorination of *C. pyrenoidosa* might potential application for bioremediation of polychlorinated biphenyls (PCBs) that with similar chemical structure to triclosan, but belonging to the catagory of persistent organic pollutants (POPs). Through the TEM observation, it was found that the triclan treatment resulted in the disruption of the chloroplast of algal cells, which indicated that triclosan may affect membrane metabolism.

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