Lipid defense response of *Chlorella* as theoretical background in wastewater treatment for pollutants

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*Chlorella* is a unicellular green alga that is used for both laboratory and applied purposes [1,2]. This alga is an effective organism for removing nitrates, nitrites and phosphates from water environments, and for indicating contamination. The original Hach Engineering Company claimed that the “Biocoil” bioreactor could remove 92% of nitrates, and 98% of phosphates from wastewater. The cell cycle of *Chlorella* in natural conditions is characterized by synchrony of reproduction with starch collection during ontogenesis. Examination by transmission electron microscopy (TEM) of *Chlorella* ontogenesis ultrastructure after co-immobilization with *Phyllobacterium* and *Azospirillum* bacteria demonstrated a strong change of alga differentiation. After some time takes place a losing of reproduction synchrony (i), collecting granules of starch (ii) and, (iii), collecting lipid granules until transformation algal cell in to adipocyte form. In this article, the existence of a lipid defense response, is proposed based on TEM study of *Chlorella* ontogenesis after co-immobilization with bacteria, which might theoretical background for explanation of the removal mechanism for lipid-dissolved pollutants from shed water.

**Keywords:** *Chlorella*; lipid synthesis; defense response; pollutants; TEM.

*Chlorella* es un alga verde unicelular usada ampliamente para el laboratorio y para propósitos prácticos [1,2]. Esta alga es un organismo eficaz para eliminar nitratos, nitritos y fosfatos de ambientes acuáticos, y como indicador de contaminación de aguas. La compañía original de ingeniería Hach proclamó que el bioreactor “Biocoil” puede remover 92% de los nitratos, y 98% de los fosfatos de aguas residuales. El ciclo celular de la *Chlorella* en condiciones naturales se caracteriza por la sincronía de la reproducción, con acumulación de almidón durante la ontogénesis. El examen ultraestructural de la ontogénesis de *Chlorella* tras su co-inmobilización con las bacterias *Phyllobacterium* y *Azospirillum* por microscopía electrónica de transmisión (MET), demuestra un fuerte cambio en el carácter de la diferenciación del alga. Después de algún tiempo, pierde sincronía en la reproducción (i), acumula granulos de almidón (ii) y acumula granulos de lípidos (iii), hasta la transformación de la célula del alga en una forma de adipocitos. En este artículo, tomando como base estudios de MET de la ontogénesis de *Chlorella* tras su co-inmobilización con bacterias, se propone la existencia de una respuesta de defensa basada en la producción de lípidos, la cual puede ser un fondo teórico para la explicación de los mecanismos de remoción de contaminantes lipo-solubles de aguas negras.

**Descriptores:** *Chlorella*; síntesis de lípidos; respuesta de defensa; contaminantes; MET.

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1. **Introduction**

The freshwater supply is becoming increasingly contaminated in many parts of the world, and the ability to purify it is not often sufficiently advanced. From an ecological point of view, sustainable development in a country cannot be achieved without an adequate supply of recycled and unpolluted freshwater.

Many communities in Mexico do not have access to safe water for drinking and cattle (as examples, only 64% of the residents of Nogales, Sonora and about 80% in Mexicali, have safe water) [2].

The water obtained during a low rainfall period cannot supply everyday water needs without cleaning it of contaminants, among which are nitrates, nitrites, phosphates, heavy metals and pesticides.

These compounds are abundant and widely dispersed in areas with agricultural and industrial activity.

Removal of pollutants from water by *Chlorella* just explained by changing in unique cell wall of alga and in the composition of it: by increase in rigidity and the lipid quantity of it cell wall and plasmalemma. Laboratory studies have shown that there are two active absorbing substances in *Chlorella* – sporopolein and alga cell wall [3,4]. The cell wall of *Chlorella* have three layers, of which the thicker middle layer contains cellulose micro fibers and surfactants that are non-polar absorbents of contaminants [5]. This of *Chlorella* cell wall structure, might explain the detoxification ability of *Chlorella*.

2. **Material and methods**

Methods of preparing samples for TEM can be found in [7].

3. **Results and discussion**

It is well known that *Chlorella* in unfavorable environment changes of its initial reproduction synchrony. The influence of some organisms (e.g., dinoflagellata, bacteria, some poison compounds) [8] in water affect on *Chlorella* ontogenesis by re-direction it carbon metabolism.
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Figure 1. Chlorella differentiation is affected by unknown bacterial factor(s); a) shows Chlorella on the first stage of starch creation; cw - cell wall, m – mitochondria, p – pyrenoid of chloroplast, St – starch, th – thylakoids; b) shows section of multicellular colony of Chlorella; chl – chloroplast, cw – cell wall, N - nucleus; c) shows lipid and starch synthesis by Chlorella cells; cw – cell wall, St-starch, L-lipids; d) shows one of the last stages of Chlorella transformation into adipocyte form.

In the study described here, Chlorella began to collect starch as usual after co-immobilization with bacteria, but all carbon pathway changed, so the algae lost their characteristic one-cellular stage and after several divisions both one-cellular and multicellular stages were observed (Figs. 1a-1d). In these stages of differentiation, Chlorella cells transformed into a special – adipocyte form with strong collection of lipids. In addition to the opinion of other authors [1,3,4], analysis of Chlorella cells ultrastructure supported our opinion that this lipid defense response may explain how alga remove and conserve lipid-solving pollutants.

Today there is no doubt about the connection between pesticides use and their harmful effects on wildlife and health of people . Pesticides are thought to cause hormonal imbalance in organisms by mimicking natural hormones, interfering with cell function and activating unusual enzyme activity [11]. Additional health risks might arise from synergistic effects of pesticides, and from increasing concentrations of pollutants in different organisms. Furthermore, all of the major pesticides in use have been implicated, possibly or certainly, as mutagens or teratogens. Most of them are extremely stable, and, as a result, they persist in the environment for a long time – some for years and more.

Both laboratory experiments and analysis of material collected in the field have shown lipid composition in organisms to have significant effect on bioaccumulation, and on lipid steady-state concentration of pollutants . Dangerous compounds such as organochlorines, tend to concentrate in living cells and tissues at the top of the food chain [5,6,11]. This raises the question: How can living organisms survive in water with pesticides, that are collected in their cells? Almost all poison compounds concentrated and dissolved in lipid media are in an inactive state, and are enclosed by membranes produced by Golgi complex. This insulates the poison compounds from other parts of cells and tissues is usually realized by secondary lysosomes , which are the “cell garbage disposal system” [9].

An analogous defense response was developed and reserved during the evolution of cells and specialized excretory tissues and organs, such as chloride cells of plants ,the chloragogenous epithelium of invertebrates, the fat body of insects, and the kidney and liver of various animals.

The redistribution of persisting organic pollutants, which occur in organisms as a consequence of lipid depot, can be studied by modeling artificially modified Chlorella ontogenesis. After several divisions, Chlorella cells have fates or options in cell differentiation to form new cell populations. Two of them are very interesting for us (Figs. 1a-1d, and 2).

Algae cells after first mitosis continue to divide, forming colonies of four- and more cells with a common cell wall, and some algae live independently (on a custom basis). In both pathways of differentiation, lipid defense response switches on by collecting starch granules and lipids until the algae transform into adipocyte forms.

It is well known that bacteria can produce various plant growth promoting hormones [11], some of which stimulate algae growth. Furthermore, it is reasonable to assume that Chlorella has the ability to take up exogenous cobalamin produced by bacteria and to synthesize coenzyme forms [10].

Figure 2. Two directions of Chlorella differentiation under unfavorable conditions. I – multicellular pathway of differentiation; II – unicellular pathway of differentiation. In both possible pathways, Chlorella transforms into adipocyte form. L – lipids. Arrows show two directions of cell differentiation.
In turn, cobalamin might stimulate lipid synthesis of *Chlorella*.

Study of *Chlorella* ultrastructure (Figs. 1a-1d, and 2) obtained by TEM shows possibility for the shed water contaminant removal mechanism. These data obtained on “lipid defense response” of Chlorella open a new theoretical background for cleaning wastewater of lipid-dissolved and poison compounds.

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