Desalination and Water Treatment
www.deswater.com

2 (2009) 101–107

1944-3994 / 1944-3986

Biosorption of nutrients by *Zygnema sterile* and *Lepocinclism textra* biomass in high rate algae culture system

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Received 30 July 2007; accepted 14 September 2007

ABSTRACT

The biosorption characteristics of N and P nutrients by *Zygnema sterile* and *Lepocinclism textra* biomasses were closely investigated using a lab scale high rate culture system. From operating this system, the following points were obtained: the calculated AGP was 4.58×10^{-3} mg/d; the maximum of DO generation was 5.8 mg DO/L during peak algal growth for 1403.97 Chl.-a μg/L; this system generated an average of 0.77 mg/L DO/d; the fundamental biosorption mechanism can be considered as the movement of (+) and (–) electric charge on the algal biomass surface, similar to one of ion exchange; with the biosorption and passage of (–) ions onto and through the cell wall, respectively, the protomotive force becomes predominant.

Keywords: Biosorption characteristics of nutrients; *Zygnema sterile* and *Lepocinclism textra* biomasses; Ion exchange; Protomotive force

1. Introduction

In the general field of biosorption, the use of living algae biomass, especially for treating wastewater containing nitrogen and phosphorous; in principle, relies on the biosorption ability of nutrients due to the proliferation of the algae cells in the water via photosynthesis and carbon assimilation [1]. At present, the dead biomass of algae (algal biopolymer) play significant roles as biosorption and recovery agents of precious and strategic metals in Canada [2]. However, that the use of algal biomass is not entirely new in the advanced treatment of wastewater, as this technology has already been operated and maintained over long periods of time, using high rate ponds (HRP), etc., especially for the purpose of nitrogen and phosphorous removals and increasing the DO content: this technology relies on the biosorption ability of algae cells as its core function. As an advanced wastewater treatment, many investigations have tested the use of specific algae biomasses (Table 1) [3], where securing cheap materials for this purpose has been regarded as important work. Especially, the necessity for the artificial mass culture of algal biomass has been proposed. With respect to this problem, it is very useful to refer the Far East Asian culture ability for *rhodophyta*, such as laver (micro algae), etc., which is popular in Korea and Japan [4]. Considering this ability, the culturing of algae populations that possess the required biological characteristics can be controlled by means of the irradiation light intensity, water temperature and supply of nutrients, such as inorganic nitrogen and phosphorous. Therefore, research relating to this ability has concentrated on confirmation of the environmental factors governing the mass culture of algae and the optimum physicochemical conditions, such as the light intensity, water temperature and concentration of nutrients, etc. [5]. Pre-

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Presented at IWA Efficient 2007, May 20–23, 2007, Jeju, Korea

Table 1
Summary of the literature on the application of immobilized algae for wastewater treatment

Algal Taxa	Waste treated	Immobilization technique	Type of culture or reactor	Developer
AlgaSORB	Metals	Silica gel	PBR	Darnall et al. (1991)
Anabaena CH_3	N	Alginate	Batch and semi-continuous	Lee et al. (1995)
Anabaena doliolum and *Chlorella* vulgaris	N, P and metals	Agar, alginate carrageenan and chitosan	Batch and semi-continuous	Mallick et al. (1993)
Aphanocapsa pulchra	Metals	Alginate	PBR	Subramanian et al. (1994)
Chlamydomonas reinhardtii	NO_3, NO_2,	Alginate	Batch and PBR	Vilchez et al. (1994)
Chlorella emersonni	P	Alginate	Batch and PBR	Robinson et al. (1994)
Chloralla emersonni	Hg	Alginate, agar and agarose	Batch and PBR	Wilkinson et al. (1992)
Chloralla homosphaera	Cd, Zn, Au	Alginate	Batch	da Costa et al. (1991)
Chlorella regularis	U	Polyacrylamide	Batch and PBR	Nakajima et al. (1982)
Chlorella vulgaris	Metals	Polyacrylamide	PBR	Darnall et al. (1986)
Chlorella vulgaris	N, P	Alginate	Batch	Tam et al. (1994)
Chlorella vulgaris, *Chlorella kessler* and *Scenedesmus quadricauda*	Cattle manure	Alginate carrageenan polystyrene polyurethane	PBR, FBR	Travsco et al. (1992)
Chlorella vulgaris and *Scenedesmus bijugatus*	N, P	Alginate	PBR	Megharaj et al. (1992)
Cyanobacteria	Zn, Mn	Mixed biofilm on glass wool	PBRs	Bender et al. (1994)
Nostoc calcicola	Cu	Alginate	Batch	Singh et al. (1992)
Nostoc calcicola	Methyl-Hg	Alginate	Batch	Pant et al. (1992)
Pharmidum sp.	Urban effluent	Chitosan	Batch and semi-continuous	Proulx et al. (1988)
Pharmidum laminosum	P	Polyvinyl foam	Batch, PBR, FBR	Garbisu et al. (1993)
Pharmidum laminosum	NO_2, NO_3	Polyvinyl and polyurethane foam	Batch, PBR	Garbisu et al. (1992)
Prototheca zoptii	Kepone	Agar	PBR	Pore et al. (1981)
Sargassum fuitans	heavy metals	Synthetic polymers	PBRs	Ramelow et al.(1996)
Scenedesmus acuta and *S. obliquus*	N, P	Carrageenan	Batch	Chevalier et al. (1985)
Scenedesmus bicellularis	N, P	Alginate and chitosan	Repeated batch	Kaya et al. (1995)
Scenedesmus obliquus	NO_3	Ipolyurethane polyvinyl	Batch and PBR	Kaya et al. (1996)
Spirulina maxima	Swine waste	Carrageenan	FBR	Urrutia et al. (1995)
Scenedesmus quardricauda	N, P	Carrageenan	Batch	Chevalier et al. (1985)

Note: N = nitrogen, P = phosphorus, PBR = packed-bed reactor FBR = fluidized bed reactor

viously, the proliferation of algal biomass has been verified, through several experiments, and found to rely on algal and bacterial symbiotic action [6], and is the mechanism most often utilized to achieve the biological purification of river and lake water ecosystems (Fig. 1). In such ecosystems, the natural metabolism for the survival of aerobic heterotrophic bacteria increases the water solubility of CO_2, NH_4^++NO_3^- and PO_4^{3-}, etc, and algae intake such nutrients, where they are converted to O_2 and H_2O [7], which are subsequently reused for the catabolism of organics by the aerobic heterotrophic bacteria.

2. Materials and methods

2.1. Experimental apparatus and method

The experimental apparatus was composed of a storage tank and 2 reaction tanks, which were made of acryl. Volumes of the storage and reaction tanks were 125 and 64 L, respectively (Fig. 2). The reaction tank was fitted with an agitator, water level controller, water temperature controller and light irradiation apparatus. The water temperature controller consisted of a thermostat, submerged heater, hot water generator and rod type ther-

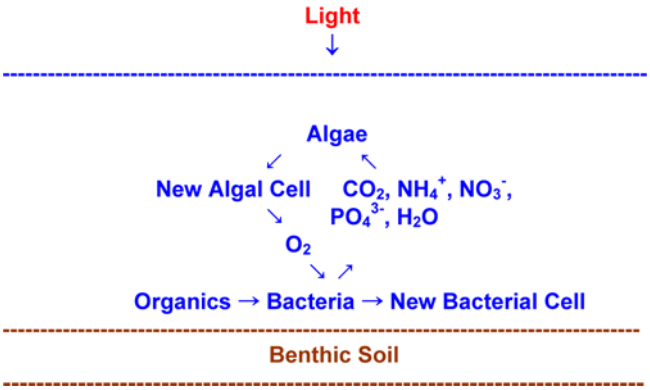

Fig. 1. Algal and bacterial symbiotic action.

of an on-off switch, 12 sets of 40 Wt and 8 sets of 20 Wt, 3 wavelength fluorescent lamps, which were installed near the reaction tanks. The water level was controlled by the use of floating valves.

In the main experiment, the preliminary algae culture test was initiated. A 64 L water sample was collected from a river water clear zone, poured into the culture medium, with the addition of nutrients (see Table 2) into same cultivator. The culture conditions consisted of: 1 month culture duration, water temperature of 25°C, agitation velocity of 50 rpm and a light intensity onto the water surface of 3000 LUX. In the next stage of the main experiment, clay powder, as an immobilization agent for entrapping the algal biomass, was place over the bottom of the reactor tank at thickness of 3 cm, with 2 sets of

Table 2
Nutrient composition added for algae cultivation

Nutrient	Chemicals	Amount (mg/L)
Macro nutrient	KNO_3	80
	K_2HPO_4	11.25
	KH_2PO_4	8.44
	$MgSO_4 \cdot 7H_2O$	250
	$CaCl_2 \cdot 2H_2O$	15.47
	$Fe_2(SO_4)_3$	4.06
	$NaHCO_3$	167.97
	Na_2EDTA	4.88
Micro nutrient	$MnSO_4 \cdot H_2O$	1.41
	$ZnSO4 \cdot 7H_2O$	0.2
	$CuSO_4 \cdot 5H_2O$	0.08
	H_3BO_3	3.13
	$(NH_4)_6Mo_7O_4 \cdot 4H_2O$	0.13
	$CoCl_2 \cdot 6H_2O$	0.04

artificially compounded wastewater (standard urban sewage; 64 L each) (Table 3) introduced into each of the reactor tanks, along with diluted algae solution (0.47 Chl.-a mg/m^3).

These mixed algae solutions were cultured, and then used in a batch style operation under the following conditions: pH 7.0, water temperature; 25°C, agitation velocity; 15 rpm, with 24 h light irradiation at a light intensity onto the water surface; 3000 Lux. The culture solu-

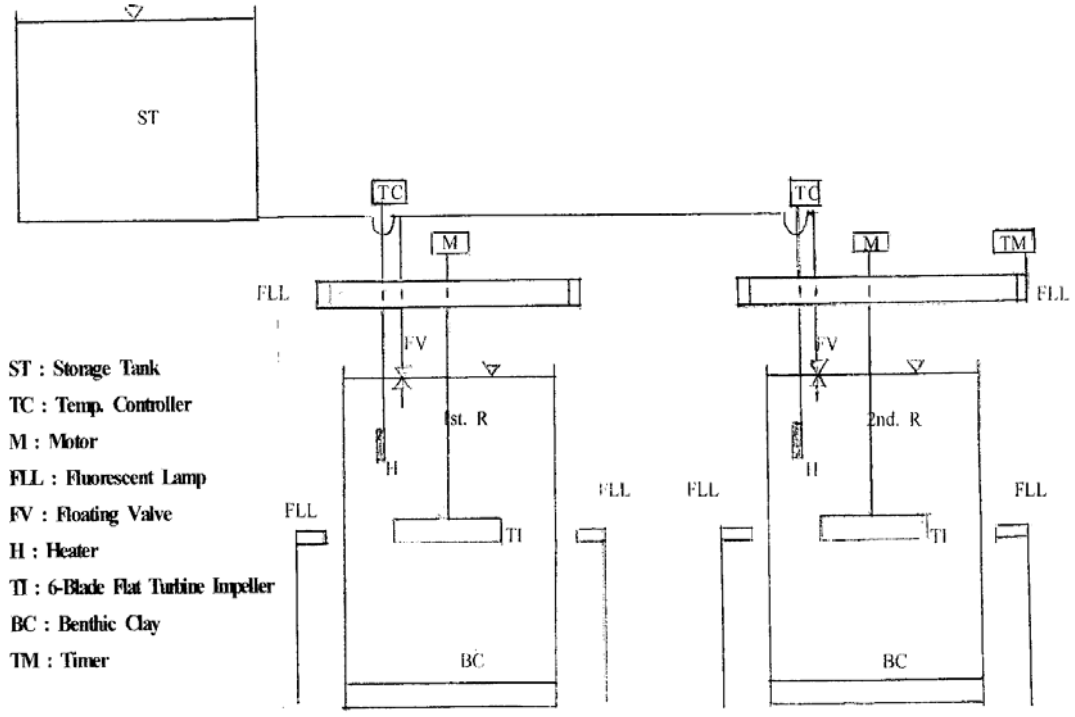

Fig. 2. Schematic diagram of pilot scale experimental apparatus.

Table 3
Composition of artificial wastewater used in main experiment

Component	Concentration(mg/L)
Starch	300 (300mg COD cr/L)
$(NH_4)_2CO_3$	102.9 (30mg NH_3-N/L)
Na_2HPO_4	41.4 (9mg PO_4-P/L)
$MgSO_4 \cdot 7H_2O$	250
$CaCl_2 \cdot 2H_2O$	15.47
$Fe_2(SO_4)_3$	4.06
$NaHCO_3$	167.97
Na_2EDTA	4.88
$MnSO_4 \cdot 5H_2O$	1.41×10?³
$ZnSO_4 \cdot 7H_2O$	0.2
$CuSO_4 \cdot 5H_2O$	0.08
H_3BO_3	3.13×10?³
$(NH_4)_6Mo_7O_4 \cdot 4H_2O$	0.13
$CoCl_2 \cdot 6H_2O$	0.04

Table 4
Analytical methods and instruments

Items	Analytical method and measuring instrument
Temperature	Thermometer
Illumination	Illuminator (Model DM-28, Takemura, Japan)
pH	pH meter
DO	DO meter (YSI model 518, Azide modification)
CODcr	US Standard method (Open reflux method)
NH_3-N	US Standard method (Phenate method)
NO_3-N	ROK Standard method (Brucine method)
PO_4-P	US. Standard method (Stannous chloride method)
Chl.-a	Spectrophotometric method (Extraction with acetone)

tion was sampled everyday, and the contents then analyzed, using the conditions and set up shown in Table 4. In particular, the chlorophyll-a, CODcr, NH_3-N, NO_3-N and PO_4-P contents were analyzed after filtering of the sample through a Whatmann GFC filter.

3. Results

The first date concentrations of NH_3-N and NO_3-N and PO_4-P and CODcr were 18.98, 1.50, 8.94 and 68.97 mg/L, respectively. This means that the adsorption by clay powder and oxidation by nitrification bacteria produced a notable decrease in the nutrient contents. As the culture continued, a strong green color developed in the cultivator after 3–4 d (adaptation period) as the chlorophyll-a concentration gradually increased (Fig. 4), with

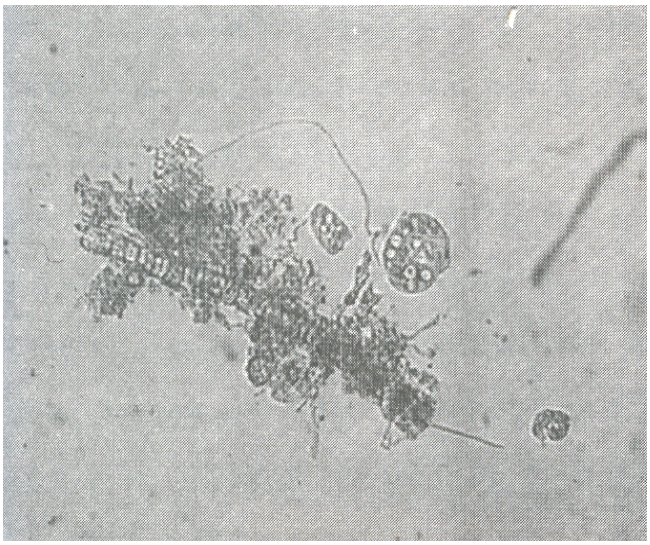

Fig. 3. *Zygnema sterile* and *Lepocinclism textra.*

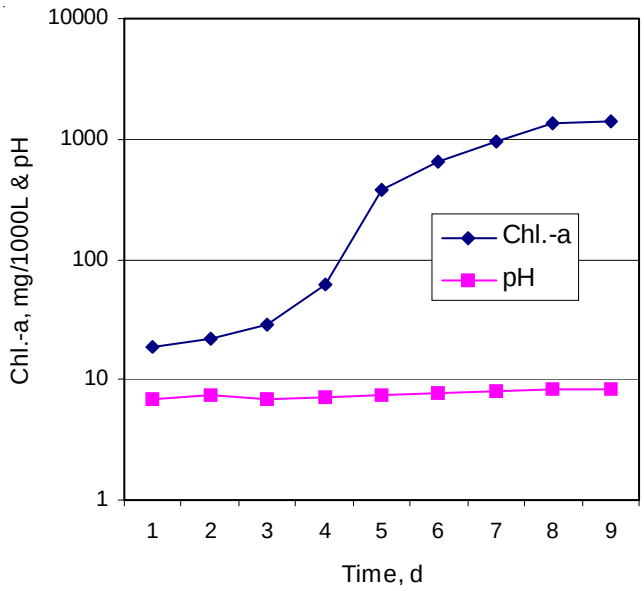

Fig. 4. Changes of chlorophyll-a, pH.

Zygnema sterile and *Lepocinclism textra* (Fig. 3), floating flagellate alga appearing as the dominant algae strains.

The trends of NH_3-N, NO_3-N, PO_4-P, CODcr and DO also changed as the *Zygnema sterile*' and *Lepocinclism textra*' grew, a indicated in Figs. 5, 6 and 7.

3.1. AGP (algal growth potential) and generation of oxygen

The algal growth can be considered the result of a 1st order biochemical reaction. Therefore, the AGP (algae growth potential) can be expressed in the form of the Michaelis-Menten equation, as follows:

$$X_t = K \cdot S \cdot S_m \cdot t \quad (1)$$

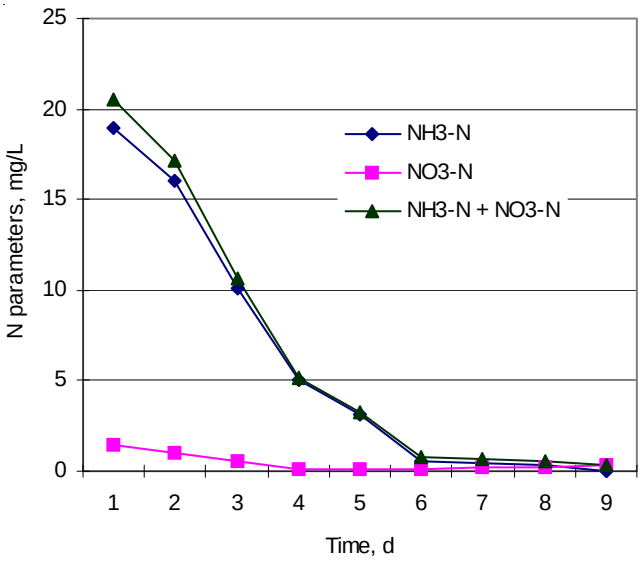

Fig. 5. Changes of NH_3-N, NO_3-N.

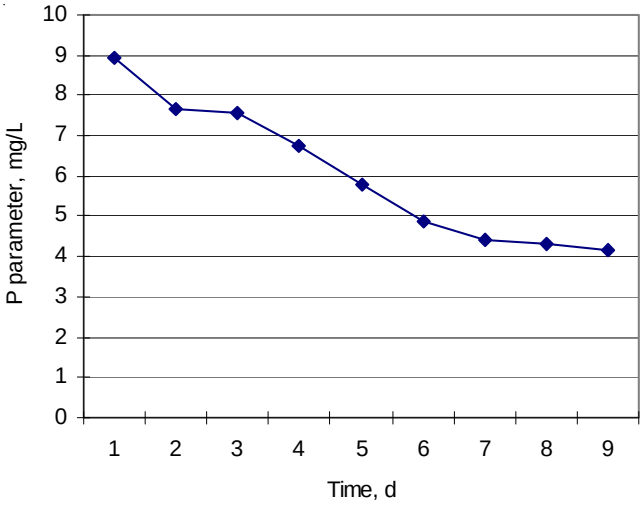

Fig. 6. Changes of PO_4-P.

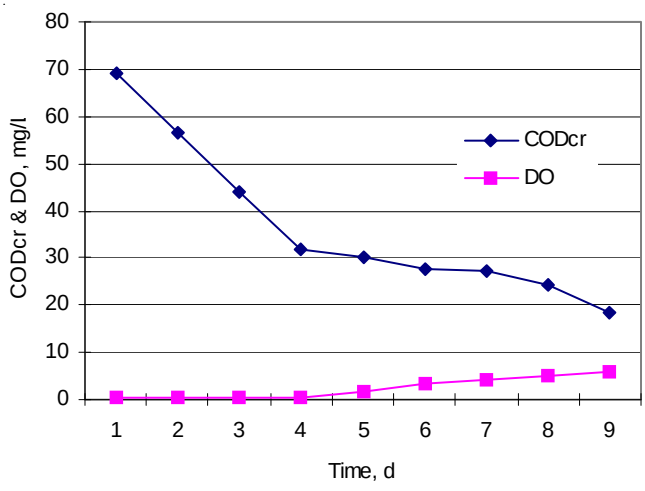

Fig. 7. Changes of CODcr, DO.

where X_t is the chl.-a concentration after t days, μg/L; K the specific growth velocity of chl.-a, mg/d^{-1}; S the N or P concentration at the inlet into the cultivator, mg/L; S_m the N or P concentration during the biochemical reaction, mg/L and t the culture time, d.

In this culture medium, the N/P mole ratio was less than 16. Therefore, the algal growth was presumed to be governed by N concentration. Using Eq. (1), with the N concentration as the governing concept, the K value of AGP was calculated to be 4.58×10^{-3} mg.d^{-1}. The oxygen generated had a maximum of 5.8 mg DO/L during peak algal growth for 1403.97 Chl.-a μg/L, indicating that the cultivator generated an average of 0.7714285 mg/L DO/d.

3.2. *Removal efficiencies of NH_3-N, NO_3-N, PO_4-P*

The removal efficiencies of NH_3-N, NO_3-N and PO_4-P were 100, 72 and 45%, respectively, during the 8 d wastewater detention time. This re-confirmed that this reactor could not overcome the problems of the long wastewater detention time and low removal efficiency of PO_4-P, which are able to be maintained by a conventional HRP (high rate pond). However, the possibility exists that they could be noticeably reduced if this wastewater was treated again in another reactor furnished the special immobilizing ability, as several important immobilization technologies have been successfully developed.

4. Discussion

Algal biomass growth is the result of active absorption due to the vitality of cells. Adsorption is only the phenomenon created on the surface of algae cell wall as the 1st step prior to absorption. Adsorption is subdivided into cationic and anionic forms. The biosorption of cationic metallic elements proceeds in two separate steps: physical sorption and chemical sorption. Physical sorption is the reversible binding phenomenon due to electrostatic forces between the algae cell wall surface and the metallic ions [8]; whereas, chemical sorption follows on from physical sorption, and proceeds in the regular order; coordination, complexation and finally chelation. The cell walls of *Chlorophyta* consist of both carbohydrates and proteins. The carbohydrates form a fibrillar skeleton [9] as the amorphous embedding matrix. As the algae culture continues, this cellulose matter becomes mucilage, which results from sulfated changes. The components of this mucilage are uronic acids (glucoronic, mannuronic and glucuronic) and sulfated polysaccharides (involving galactose, arabinose, xylose, rhamnose, glucuronic acid). Of the several organic groups in carbohydrate, only the carboxyl (–COOH) and sulfonate (–SO_3H) groups contribute to the formation of metallic ligands. In the case of proteins, the amino acid organic groups, which are carboxyl, sulfonate, sulfhydryl (–SH), hydroxyl (–OH), phosphonate (–P(O)$(OH)_2$), thiseter (=S),

secondary amine (=NH), imine (=NH), contribute to the formation of metallic ligand [10]. Proteins, as enzymic catalysts, are also chief components of the ECP (extra cellular polymer), and the nutrients chelated by this ECP pass through the algal cell wall. Metal ligands are bound by various covalent and ionic bonds in aqueous culture media, with the extent of bondage based on the different electrostatic charges. In particular, metal chelates are often formed by the substitution of water molecules. In solution, chelating anions are proton acceptors, and protons will compete with metal ions for the anions [11], which can be represented as:

$$Me^{2+} + 2HL \Leftrightarrow [MeL_2] + 2H^+ \quad (2)$$

where, HL is protonated ligand.

With respect to the overall metal binding, the most important mechanisms are based on sorbate/solvent interactions, which in turn rely on some combination of covalent, electrostatic and Vander Walls forces. The ability for biochemical biosorption is stronger than that of physical biosorption, and the ability of physical biosorption decreases further with increases in water temperature.

The biosorption of anionic ions can be regarded as originating from the absorption by algae cells. Therefore, the kinetic constant can be easily obtained from the Michaelis-Menten formula. However, a recent investigation [12] indicated that the kinetic constants for anionic ions were merely the result of ion exchange between the algae cell wall surface and the anionic ion.

4.1. Protomotive force

The protomotive force is responsible for the transportation of anionic ions into the inside of algae cells. According to Kim [13], this protomotive force is an electrostatic force created on algae cell walls due to a conversion mechanism between ATP (adenosine triphosphate) and ADP (adenosine diphosphate), which originates from cellular nucleic metabolism.

During this metabolic process, the oxidation and reduction of orthophosphates have the enzymes (F_1F_0ATPase etc.) of protoplasm membrane primarily emit H^+ (proton) toward the outer surface of the cell wall [14], creating a force that allows the ions to pass through the membrane of the cell wall.

4.2. Modelling of biosorption

In this system, the Langmuir equation can be used for modelling of biosorption of nutrients. Assuming all of the binding sites on the sorbent are free, the equation can be written as:

$$\frac{[BM]}{[B][M]K} = ① \quad (3)$$

where K is equilibrium constant; B — free binding sites; M — sorbate in solution.

According to the mass conservation of binding sites, the total binding capacity, B_t, can be written as:

$$[Bt] = [B] + [BM] \quad ② \quad (4)$$

Combining Eqs. (3) and (4) gives:

$$\frac{[Bt]K[M]}{1 + K[M][BM]} = q = ③ \quad (5)$$

where $[BM] = q$ = sorbate uptake.

In the biosorption of nutrient ions by algal biomass, the selectivity coefficient for ion exchange can be described as:

$$K_d = \frac{\left(\dfrac{\text{ion concentration}}{\text{unit weight of algal biosorbent}}\right)}{\left(\dfrac{\text{ion concentration}}{\text{volume of solution}}\right)} \quad ④ \quad (6)$$

4.3. Development of immobilization technology

Kierstan and Bucke [15] described the formation of 4 mm diameter beads, using 5% (w/v) sodium alginate and 0.3 M $CaCl_2$, and it was initially proposed that alginate could be used as an effective immobilization agent. Thereafter, Bold and Wynne [16] also proved experimentally that the proteinaceous cuticle of Uglophyta could become an effective immobilization agent. Chevalier et al. [17] successfully performed tests on the biosorption of PO_4^{3-}, using a Chrolella strain entrapped with K-carrageenan. Furthermore, Lau et al. [18] showed that 95.2 and 100% of NH_4^+-N and PO_4^{3-}-P could be removed within 2 d using high stocking technology with an algal cellular density of 18×10^6 cell/mL: this technology is the result of using a 5% (w/v) aqueous solution of K-carrageenan extracted from *Eucheuma cottoni*. Many other investigations are continuing to experimentally confirm that algae cells proliferate well in such fibrous gel solutions, and also satisfactorily remove anionic nutrients, such as NO_3^--N and PO_4^{3-}-P, etc.

5. Conclusion

As a result of this study, the following conclusions were obtained.

1. The following experimental data were obtained from this artificial algae culture system.
   - *Zygnema sterile* and *Lepocinclism textra* growth potential: 4.58×10^{-3} mg/d^{-1}
   - Maximum generation of oxygen: 5.8 mg DO/L during peak growth of 1403.97 Chl.-a μg/L for the above algae
   - The mean DO generated: 0.7714285 mg/L/d average value.

2. With the biosorption and passage of (–) ions onto and through the cell wall, respectively, the protomotive force becomes predominant.

3. The biosorption of nutrients, such as N and P, etc. onto the algal cell surface is simply a fundamental mechanism of the movement of (+) and (–) electric charges, similar to that of ion exchange resins.

4. The above mentioned facts imply that a well developed algae biomass biosorbent could be used for the efficient removal of nutrients from sewage, such as N and P, etc.

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