Southeast-Asian J. of Sciences: Vol. 2, No 1 (2013) pp. 41-51

AMMONIA UPTAKE BY UNICELLULAR GREEN MICROALGAE: MATHEMATICAL MODELING AND PARAMETER OPTIMIZATION

Artorn Nokkaew[1], **Wannapong Triampo**[2],
Somkid Amornsamankul[3], **Busayamas Pimpunchat**[4],
Charin Modchang[5] and **Darapond Triampo**[6]

[1] *Institute for Innovative Learning*
Mahidol University, Nakorn Prathom 73170, Thailand
e-mail: krooart@gmail.com

[2] *Dept. of Physics, Mahidol Univ., Bangkok 10400, Thailand*
Ins. for Inno. Learning, Mahidol Univ., Nakorn Prathom 73170, Thailand
e-mail: scwtr@mahidol.ac.th, wtriampo@gmail.com

[3] *Dept. of Math., Faculty of Science, Mahidol University*
Center of Excellence in Mathematics, Bangkok 10400, Thailand
e-mail: somkid.amo@mahidol.ac.th

[4] *Industrial Mathematics Research Unit and Department of Mathematics*
King Mongkut's Inst. of Tech. Ladkrabang, Bangkok 10520, Thailand
e-mail: knbusaya@kmitl.ac.th

[5] *Dept. of Physics, Mahidol Univ., Bangkok 10400, Thailand*
e-mail: sccharin@mahidol.ac.th

[6] *Dept. of Chemistry, Mahidol Univ., Bangkok 10400, Thailand*
darapond.tri@mahidol.ac.th

Abstract

We provide a system of differential equations to model nitrogen consumption of a unicellular green microalgae and their growth. The model was modified to account different mechanisms of unionized ammonia and ammonium ion, up taken by unicellular green microalgae. An agreement between the theoretical mathematical model and experimental measure-

---

Corresponding author: Wannapong Triampo.

Key words: ammonia uptake, genetic algorithms, optimization, ordinary differential equations, parameter estimation.

ments was studied in order to validate the model. Parameter optimizations were performed by using a genetic algorithm.

1 Introduction

In the aquatic environment, nitrogen is essential for living of phytoplankton. In some cases, it can act as a limiting factor of their production. Microalgae can take up some forms of nitrogenous compounds (ex: nitrate and ammonia) as a precursor for amino acid and nucleotide synthesis. Consequently, inorganic nitrogen compounds in environment decrease. Simultaneously, yields of microalgae increase. On one hand, microalgae can be utilized for water treatment. On the other hand, taking up of wastes by algae results gain of algae biomass which is a source of commercially significant products, such as biofuel and source of nutrient supplements.

The main nitrogen sources for microalgae include nitrate, nitrite or ammonia. However, reduction of the oxidized forms of nitrogen requires the expenditure of metabolic energy [3]. So, ammonia is more preferable than nitrate or nitrite. When both nitrate and ammonium ion are available, ammonium ion is used first and NO_3 uptake is inhibited [5].

The term ammonia in this paper refers to two chemical species: unionized and ionized. Normally, tests for ammonia measure total ammonia $(NH_3 + NH_4^+)$. Making more specific, term unionized ammonia refers to NH_3 and ammonium ion or unionized ammonia will refer to NH_4^+.

Ammonia contaminated in environment originates from sewage discharged, effluent from domestic and also organism excretion. Ammonia is very soluble in water (Henry 's law constant is about 60 $mol \cdot kg^{-1} \cdot atm^{-1}$ at $25°C$). When ammonia is dissolved in water it will react with hydrogen ions to form ammonium ions:

$$NH_3 + H^+ \longleftrightarrow NH_4^+ \tag{1}$$

However, the reaction is reversible. Thus, ammonia exists in water in both ionized and unionized forms. Equilibrium exists in water between the quantity of unionized (NH_3) and ionized (NH_4^+) forms. The relative concentrations of NH_4^+ and NH_3 are pH dependent. The pK_a of NH_3/NH_4^+ is around 9.3 at $25°C$ [3]. Once in an acidic solution, ammonia quickly forms NH_4^+ by reaction (1). Below pH 8 there is almost exclusively ammonium ion, but above pH 10.5 it becomes almost entirely unionized ammonia (p. 41-42) [10]. The reaction also depends on temperature. As pH or temperature rises, NH_3 increases relative to NH_4^+. In nature, both unionized ammonia and ammonium ion exist.

Unionized ammonia and ammonium ion can be assimilated by algae through different pathways due to difference of their properties. Expressed in non-ionized form, ammonia is uncharged and moderately lipid soluble. NH_3 is readily permeate through membranes. Unlike unionized ammonia, ammonium ion is charged and hydrated. There are efficient transport systems specific for NH_4^+ [19],[16]. Eukaryotic algae is able to assimilate actively ammonia only in ion form [16],[2].

Ammonium ion is transported into cell through a specific channel [19],[16]. The transportation involves an energy-dependent uptake mechanism. NH_4^+ is the form that cell can use for growth. Significant intracellular ammonia pools ($\sim 1-10$ mol $\cdot\, m^{-1}$) are essential for the synthesis of amino acids from ammonia [15]. Within cell chloroplast, ammonium ion is incorporated into amino acids, catalyzed by glutamine synthetase and glutamate synthase cycle [17]. NH_4^+ subsequently diffuses across the cytoplasmic membranes back into the medium in the form of NH_3 [13].

Unlike ammonium ion, unionized ammonia is uncharged. NH_3 were generally considered to be highly permeable across biological membranes directly through the lipid bilayer. NH_3 penetrates into cell through simple diffusion. Thus, the membrane NH_3 permeability can be calculated from Ficks first law. The ammonia permeability of $(1.3 \pm 0.4)10^{-1} cm \cdot sec^{-1}$ at T = $25°C$ was reported by [20]. NH_3 is readily diffusible not only via the lipid bilayer but also through aquaporins and other more specific porins [15]. The rate of unionized ammonia uptake not only depends on the diffusion rate through the cell wall, but also on the difference between the concentration inside and outside the cell [11]. Unionized ammonia diffusion through membrane depends on ambient and also internal unionized ammonia concentration. At high ambient unionized ammonia concentrations, the passive diffusion of NH_3 into cell can take place. Consequently, High external unionized ammonia concentration corresponds to high unionized ammonia in this organism. The high permeability of NH_3 can overwhelm any transport of the NH_4^+. However, as the ambient unionized ammonia concentration is reduced and active ammonium ion transport is needed necessarily to sustain cell growth [8].

In aerobic cells, typical internal cellular pH is around 7.3 [15]. Even through there is some assumptions that, probably, diffusion of NH_3 causes an increase in pH within cells [1]. It is evident that microalgae can maintain intracellular pH in an optimal range. C. vulgaris cytoplasmic pH is maintained at value 7.2 – 7.6 at external pH of either 3 or 10. At this pH level, once unionized ammonia crosses the membrane, it may associated with a proton to form ammonium ion depending upon the pH which may slightly vary within the matrix of the cell [4]. Consequently, ammonia in the cell is mainly ionic NH_4^+ because intracellular pH is far below the pK_a of $NH_3/\ NH_4^+$ (pH $\ll$ pK_a; ca. 9.3).

The relative amount of unionized ammonia and ammonium ion is determined by the pH of the solution. The ratio of $NH_3/\ NH_4^+$ can be calculated from the Henderson-Hasselbach equation:

$$pH = pK + log(NH_3/NH_4^+) \tag{2}$$

Using the equation (2), the ratio of NH_3 / NH_4^+ can be calculated at the initial pH of each culture.

Unionized ammonia is assumed to be toxic species. It is evident that the system is more inhibitory as the ratio of NH_3/NH_4^+ in culture medium increases [4]. Unionized ammonia can diffuse readily through the plasma membrane and inner membrane of organelles. Penetrating unionized ammonia causes swelling and osmotic lysis of the cells. Also, photosynthesis inhibition by ammonia was found in some species [1]. It is evident that the toxic effect varies significantly with the pH of the culture media. Greater toxicity had been observed as the pH of the media is increased [4]. At some pH values, a threshold concentration was required before toxicity was observed.

Toxicity is specie-dependent. Previous study showed toxicity of NH_3 toward C. vulgaris growth [7]. Unionized ammonia NH_3 decelerate growth of C. vulgaris.

Accounting for toxicity of unionized ammonia, differentiation between the two forms has to be accounted. In fact, the toxicity of ammonia is usually described only in terms of unionized ammonia. The model was constructed by differentiating two species of ammonia, namely unionized ammonia and ammonium ion, up taken by unicellular green microalgae. The model describes its effect to growth of microalgae.

2 Model construction

With the ambient concentration of limiting nutrient (S) and the biomass concentration (B), a simple dynamic model for microalgae in nitrogen-limited in batch culture may be formulated:

$$\frac{\mathrm{d}S}{\mathrm{d}t} = \rho \cdot B \tag{3}$$

$$\frac{\mathrm{d}B}{\mathrm{d}t} = \mu \cdot B - D \cdot B \tag{4}$$

where S is the ambient concentration of limiting nutrient $(mg \cdot l^{-1})$, B is biomass concentration $(mg \cdot l^{-1})$, ρ is the specific uptake rate (d^{-1}), μ is the growth rate (d^{-1}) and D is death rate (d^{-1}). The equation (3) describes the change of limiting nutrient concentration according to time. The equation (4) describes the change of biomass concentration according to time.

Considering toxicity of unionized ammonia, differentiation between the two forms has to be accounted. Total ammonia is discriminated into two species: ammonium ion, and unionized ammonia. Thus, three components play in this system, including ambient ammonium ion, ambient unionized ammonia and biomass. Micro algae takes up ambient ammonia species with different mechanism. Thus, their uptake rates are described separately. In this system, nitrogen which is considered as a limiting nutrient is mainly in ammonia nitrogen form. So, we consider the change of ammonia instead. The rate of change of total ammonia (TA) is considered as summation of rate of unionized and ionized amonia described as follows:

$$\frac{\mathrm{d}[TA]}{\mathrm{d}t} = \frac{\mathrm{d}[NH_3]}{\mathrm{d}t} + \frac{\mathrm{d}[NH_4^+]}{\mathrm{d}t} \tag{5}$$

Apply the equation (3),

$$\frac{\mathrm{d}[NH_3]}{\mathrm{d}t} = -\rho_{[NH_3]} \cdot B \tag{6}$$

$$\frac{\mathrm{d}[NH_4^+]}{\mathrm{d}t} = -\rho_{[NH_4^+]} \cdot B \tag{7}$$

where $\rho_{[NH_3]}$ and $\rho_{[NH_4^+]}$ are specific uptake rates of $[NH_3]$ and $[NH_4^+]$, respectively.

The model is specified based on assumptions. In this case, the rate at which an algae cell takes up a nutrient from solution in aquatic environment is controlled by the concentration of a single limiting nutrient which is ammonia nitrogen. Empirically, ammonium ion removed fits hyperbolae curve obeying Mihaelis-Menten kinetics [18]. The rate of uptake can be described quantitatively by an expression of the Michaelis-Menten model as follows

$$\frac{\mathrm{d}[NH_3]}{\mathrm{d}t} = -\rho_{[NH_3]}^{max} \cdot \frac{[NH_3]}{k_{ha} + [NH_3]} \cdot B \tag{8}$$

$$\frac{\mathrm{d}[NH_4^+]}{\mathrm{d}t} = -\rho_{[NH_4^+]}^{max} \cdot \frac{[NH_4^+]}{k_{hb} + [NH_4^+]} \cdot B \tag{9}$$

where $\rho_{[NH_3]}^{max}$, $\rho_{[NH_4^+]}^{max}$ are maximum specific uptake rates. k_{ha} and k_{hb} are limiting nutrient concentrations at half of maximum uptake rate of NH_3 and NH_4^+ respectively.

Substitute the equation (8) and (9) in equation (5),

$$\frac{\mathrm{d}[TA]}{\mathrm{d}t} = -\rho_{[NH_3]}^{max} \cdot \frac{[NH_3]}{k_{ha} + [NH_3]} \cdot B - \rho_{[NH_4^+]}^{max} \cdot \frac{[NH_4^+]}{k_{hb} + [NH_4^+]} \cdot B \tag{10}$$

When ammonia is taken up, NH_4^+ is utilized for productions. Contrarily, NH_3 acts as a growth inhibitor. In order to account toxicity effect of NH_3, the Andrew equation is used to represent the specific growth rate, associated to an ammonia inhibition function as presented in [12]. The specific growth rate can be expressed as follow:

$$\frac{\mathrm{d}B}{\mathrm{d}t} = \mu_{max} \cdot (\frac{[NH_4^+]}{K_c + [NH_4^+] + \frac{[NH_4^+]^2}{K_i}}) \cdot (\frac{K_i}{K_i + [NH_3]}) \cdot B - D \cdot B \qquad (11)$$

where K_c and K_i are the half-saturation coefficient for NH_4^+ and the inhibition coefficient, respectively.

3 Model parameter estimation

In order to validate the model, the deterministic models, equation (10) and (11) were applied to an experiment conducted by [9]. The experimental data described changes of ammonia concentration taken up by a unicellular green microalgae, Chlorella vulgaris, which results to growth of the algae. The system in the study contained nitrogen $(7.7 \pm 0.19\ mg \cdot l^{-1})$ in form of unionized ammonia and ammonium ion. The experiment provided changes of total ammonia concentration, biomass concentration and pH corresponding to time. The concentration of NH_3 and NH_4^+ versus time were generated by using the equation (2).

Genetic algorithm has been applied for determining the parameters of algae growth and uptake model. A genetic algorithm (GA) is a stochastic global optimization algorithm based on the evolutionary process [6]. Its algorithm was derived from biological concepts, namely genetic and evolution. In this case, GA was used to search for an optimal numerical value of model parameters in order to fit the model to the experiment. Setting an objective function is crucial in GA optimization. It can affect the performance of search. Based on GA mechanism, the feasible candidates will be judged either to survive or to be omitted depending on the fitness value assessed by the objective function, called fitness function.

Thus, in order to improve the search of optimal parameter values, the objective functions were investigated. The development of objective functions will rely on the goal of the search and the purposes of getting the most benefits of the data in hand.

In this study, two sets of experimental data were provided in order to fit the model. The deviations of the two data sets should be minimized. The objective function was written in form of the least squares of errors (LSE) as shown.

$$LSE = \sum_{i=1}^{2}\sum_{j=1}^{10}([\frac{D_{exp}^{i,j} - D_{sim}^{i,j}}{D_{exp}^{i,j}}]^2 \cdot w_i) \qquad (12)$$

where D_{exp} is experimental data, D_{sim} is the data obtained from solving differential equation (10) and (11) with obtained parameters, and w_i is weight

Table 1: Optimal model parameters obtained from GA

$\rho_{NH_3}^{max}$	$k_h a$	$\rho_{NH_4^+}^{max}$	$k_h b$	μ_{max}	K_i	Death rate
1.70×10^{-5}	8.509	43.375×10^{-3}	2.238	1.259	4.760	3.35×10^{-3}

of each data deviation. i is a number of data set and j is number of data points. In this case, i is set of 2 because two data sets are available, namely total ammonia concentration and biomass concentration.

Besides the normal form of least squares of errors, the additional data can be combined into the objective function in order to improve the search. The addition might be combined on the purpose of defining the searching region [14] . However, this defined region is flexible and negotiable during the search to meet a certain predefined satisfaction.

In this paper, the objective function was elaborated. Since the model was made simple, Only the concentrations of nitrogen and biomass are state variables. So, other conditional parameters were blindly embedded in the model. However, if available, the additional data can be used in order to improve the search and make the outcomes more valid. In this case, pH data at times were used to control the search indirectly. In the other word, the outcome concentrations of nitrogen were transformed to pH by the equation (2) in order to examine the correspondence of pH and nitrogen concentration. This is on the assumption that the valid change of the nitrogen concentration should corresponds to the empirical change of pH.

The addition of pH deviation measure made the objective function becomes multi objective problems. In this study, the multiple objectives are scalarized into a single objective. the elaborated objective function is expressed as

$$Objective function = LSE + \mid pH_{exp} - pH_{sim} \mid \cdot w_3 \tag{13}$$

where pH_{exp} is pH derived from experimental data, pH_{sim} is the data obtained from calculation, and w_3 is the weight of pH deviation. The optimal weights were investigated. The optimal weight of w_1, w_2 and w_3 are 1, 100 and 1 respectively. The aim of the search is to minimize the objective function. The solution which return the least squares of errors will be chosen as an optimal values for the determined parameters. The computation were conducted under the MATLAB environment. The optimal parameter values are presented in Table 1.

Graphs of the predicted data and the experimental data are plotted in Figure 1. The plot shows that simulated curves of both ammonia nitrogen and biomass concentration pass through sample data points. In other words, the parameters optimized by GA produce a model that reflects the actual chemical phenomena.

The effects of the objective functions, the equation (12) and (13), on GA performance were further comparatively studied. It was found that the parameter values returned from the two objective functions were not so far different. Only some parameters still fluctuate. In the other word, the parameters did not correlate to the fitness values. It implies that either GA did not reach to the global optimum or they are not independent. See Figure 2, the left column shows the results of GA with elaborated objective function, equation (13), whereas the right one is the results of GA with LSE objective function, equation (12). K_1, K_{ha} and d seemingly fluctuate in both objective functions (plots of d are omitted from the figure).

However, the better performance on the elaborated objective function was evidently found for the parameter μ_{max} and K_i. They smoothly approaches a certain value as the fitness value drops. Whereas, the parameter obtained by GA with LSE seem scattered. For parameter K_2 and K_{hb}, both objective functions present a certain value approaches. This implies that the values are independent and likely to be optimal.

4 Conclusion

A model of uptaking ammonia by microalgae was proposed. The model was developed in order to differentiate unionized ammonia and ammonium ion in growth mechanism. The microalgae utilizes ammonium ion for biomass production. The unionized form of ammonia is toxic. An agreement between the theoretical mathematical model and experimental measurements was studied in order to validate the model. Parameter optimizations were performed by using a genetic algorithm. Graphs simulated from obtained parameters show agreement with the experimental data. pH-elaborated objective function is comparatively better in terms of performance than the normal least squares of errors did in growth model parameter optimization.

5 Acknowledgments

The first author would like to thank The Institute for the Promotion of Teaching Science and Technology (IPST) for a scholarship. Also, this work is partially supported by Centre of Excellence in Mathematics, The Commission on Higher Education, Thailand.

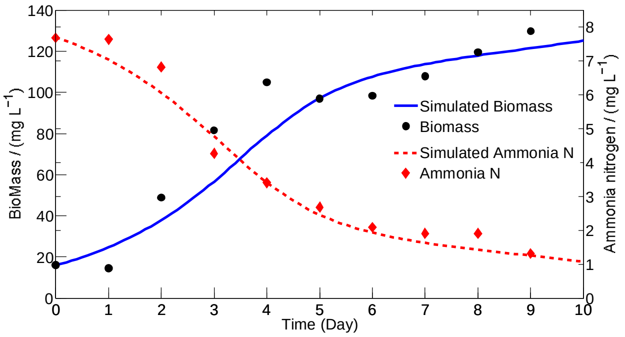

Figure 1: Plots of experimental data and simulated curves of Nitrogen concentration in ammonia form and biomass versus time

References

[1] A. Aleliovich and Y. Azov, *Toxicity of ammonia to algae in sewage oxidation ponds.*, Appl. Environ. Microbiol., 32 (1976), 801-806.

[2] L. Barsanti and P. Gualtieri, *Algae*, CRC Press (2005).

[3] C.E. Boyd and C.S.Tucker, *Pond Aquaculture Water Quality Management*, Kluwer Academic Publisher (1998).

[4] C. Doyle and M. Butler, *The effect of pH on the toxicity of ammonia to a murine hybridoma* , J. Biotech., 15 (1990), 91-100.

[5] K. Flynn and M.J.R. Fasham, *A short version of the ammonium-nitrate interaction model*, J. Plankton Res., 19 (1997), 1881–1897.

[6] D. Goldberg, *Genetic Algorithms in Search, Optimization and Machine Learning*, Addison-Wiley Publishing Company (1989).

[7] M.L. Jeong, J.M. Gillis and J.-Y. Hwang, *Carbon dioxide mitigation by microalgal photosynthesis,* Bull. Korean Chem. Soc.l, 24 (2003), 1763-1766.

[8] M. Kim, Z. Zhang, H. Okano, D. Yan, A. Groisman and T. Hwa, *Need-based activation of ammonium uptake in Escherichia coli*, Mol. Syst. Biol., 8 (2012), 1-10.

[9] J. Kim, B.P. Lingaraju, R. Rheaume, J.-Y. Lee and K.F. Siddiqui, *Removal of Ammonia from Wastewater Effluent by Chlorella Vulgaris*, Tsinghua Sci. Technol., 15 (2010), 391-396.

[10] W. Lampert and U. Sommer, *Limnoecology*, Oxford University Press Inc (2007).

[11] K. Larsdotter, *Wastewater treatment with microalgae - a literature review*, VATTEN, 62 (2006), 31-38.

[12] F. Mairet, O. Bernard, M. Ras, L. Lardon and J.P. Steyer *A Dynamic Model for Anaerobic Digestion of Microalgae* , 18th IFAC World Congress, 18 (2011), 5034-5039.

[13] T. Mller, B. Walter, A. Wirtz and A. Burkovski, *Ammonium Toxicity in Bacteria* , Curr. Microbiol., 52 (2006), 400-406.

[14] A. Nokkaew, B. Pimpunchat, C. Modchang, S. Amornsamankul, W. Triampo and D. Triampo, *Estimation of Algae Growth Model Parameters by a Double Layer Genetic Algorithm*, WSEAS Trans. Comput., 11 (2012), 377-386.

[15] R.J. Ritchie, *The Ammonia Transport, Retention and Futile Cycling Problem in Cyanobacteria*, Microb Ecol., (2012).

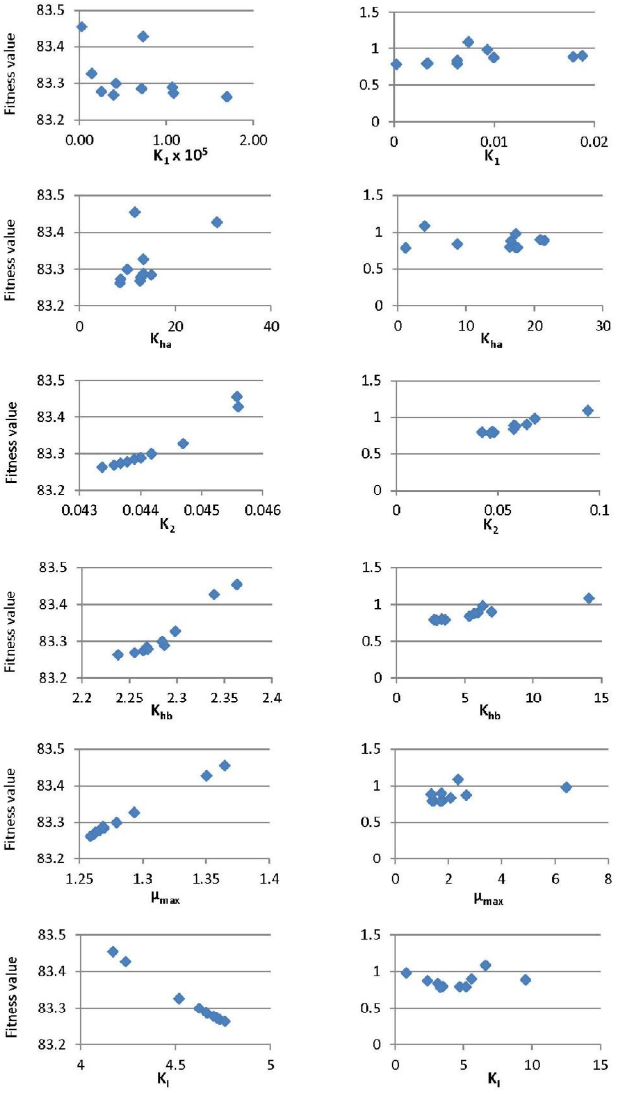

Figure 2: Plots of final parameter values obtained versus final fitness values. The left column presents the results from a GA with elaborated objective function. The right presents the results from a GA with LSE

[16] J. Schlee and E. Komor, *Ammonium uptake by Chlorella* , Planta, 168 (1986), 232–238.

[17] C. Vilchez, I. Garbayo, M.V. Lobato and J. Vega, *Microalgae-mediated chemicals production and wastes removal*, Enzyme Microb. Technol., 20 (1997), 562–572.

[18] J. Vymazal, *Ammonium uptake and biomass interaction in Cladophora glomerata (Chlorophyta)* , Br. Phycol. J., 22 (1987), 163–167.

[19] N.A. Walker, M.J. Beilby and F.A. Smith, *Amine uniport at the plasmalemma of charophyte cells: I. Current-voltage curves, saturation kinetics, and effects of unstirred layers*, J. Membr. Biol., 49 (1979), 21-55.

[20] A. Walter and J. Gutknecht, *Permeability of small nonelectrolytes through lipid bilayer membranes*, J. Membr. Biol., 90 (1986), 207–217.