*Desalination and Water Treatment*
www.deswater.com
doi: 10.1080/19443994.2013.791768

51 (2013) 6632–6640
October

Taylor & Francis
Taylor & Francis Group

# A generic stoichiometric equation for microalgae–microorganism nexus by using clarified domestic wastewater as growth medium

Maung Thein Myint[a,*,1], Abbas Ghassemi[a,b], Nagamany Nirmalakhandan[c]

[a]*Institute for Energy and the Environment, New Mexico State University, MSC WERC, P.O. Box 30001, Las Cruces, NM 88003, USA*
*Tel. +1 5756462073; Fax: +1 575 6465474; email: mmyint@nmsu.edu*
[b]*Chemical Engineering Department, New Mexico State University, MSC ChE, P.O. Box 30001, Las Cruces, NM 88003, USA*
[c]*Civil Engineering Department, New Mexico State University, MSC 3CE, P.O. Box 30001, Las Cruces, NM 88003, USA*

Received 8 August 2011; Accepted 30 January 2013

ABSTRACT

Stoichiometric equations for microalgae–microorganism nexus culture were established by using 18 sets of experimental data from the literature where clarified domestic wastewater (CDWW) was used as a growth media. The best correlations between observed and calculated coefficients in the equations were 0.908, 0.878, 0.838, and 0.849 for reactors $R_1$, $R_2$, $R_3$, and the sum of three reactors, respectively. Then, each stoichiometric equation was generalized to quantitatively describe the reactions in each set of data for 18 different experimental conditions. By analysis, the most and second most sensitive limiting parameters were identified from the 18 stoichiometric equations. The formulas and equations were further upgraded to a generic form for a general organic growth medium ($C_aH_bO_cN_dP_f$). Microalgae–microorganism nexus was established by using the developed coefficients to depict the limited and balanced reactions. The study showed that nitrogen and phosphorus are required as additives in CDWW in order to gain a balanced microalgae–microorganism nexus for a higher yield of microalgae and microorganisms. For a balanced microalgae–microorganism nexus, the optimal nutritional molar ratio of C, N, and P in the CDWW growth medium was found to be 53:15:1.

*Keywords:* Microalgae; Microorganism; Nitrogen; Phosphorous; Stoichiometric

## 1. Introduction

A model or stoichiometric equation is crucial in predicting the most sensitive parameter from a set of process influence parameters [1,2]. With the assistance of experimental data from the literature, a model or stoichiometric equation can be used in preliminary studies ahead of small lab scale research experiments to reduce time, efforts, and cost of the laboratory-scale studies and to improve the trial-and-error method of

*Corresponding author.

[1]Present address: Civil Engineering Department, New Mexico State University, MSC 3CE, Las Cruces, NM 88003, USA. Tel. +1 5753129300; Fax: 1 575646 6049.

research practice. For example, optimal molar ratio of carbon, nitrogen, and phosphorus developed from stoichiometric equations could be used to design laboratory, pilot- and field-scale microalgae production using cheap organic waste substrate thus reducing operational cost, demand for physical labor, and time. In a different study, Fisher and Huber-Lee [3] applied an improved model's capacity to predict sustainable management, infrastructure planning, and conflict resolution.

The stoichiometric equations are used to optimize methane energy gas production and to gain a higher quality methane gas in the biological processes. The balanced substrates and macro- and micro-nutrients in the Acadja reactor demonstrated that a higher quality algal biomass, which consisted of 50 kg of available total organic biomass. This value is 5 times higher than the biomass content observed in open lagoon water [4].

To produce every kilogram of dry microalgae, 20.3 L of water, 134 g salt, 147 g nitrogen, and 20 g phosphorus are required [5]. For a sustainable and cost-effective process, the growth medium-containing carbon, nitrogen, phosphorus, micronutrients, and water have to be reused from the waste such as clarified domestic wastewater (CDWW) [6].

However, the microalgae culture becomes complex when CDWWs is used as a growth medium as it contains microorganisms. In this respect, the microorganisms here are defined as all the microbial lives that naturally exist and grow in CDWW. During the growth, microalgae generate molar dissolved $O_2$, which is required for aerobic degradation of organic contaminants, and to fix the dissolved $CO_2$, that is, a product of respiration (Figs. 1(a) and 1(b)). Both microalgae and microorganisms consume carbon, nitrogen, and phosphorus from CDWWs elementally characterized as $C_aH_bO_cN_dP_f$. Microalgae degrade CDWW by using light and release dissolved $O_2$ into the growth medium. Microorganisms degrade CDWW by using dissolved $O_2$ from the growth medium and concurrently with the microalgae process, release dissolved $CO_2$ into the growth medium. The dissolved

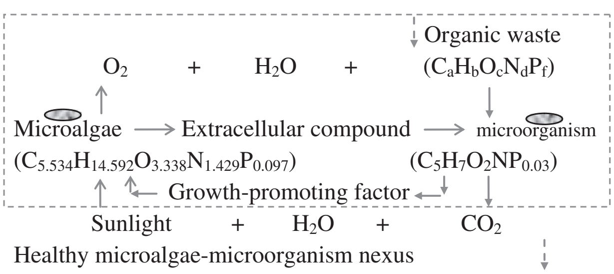


Fig. 1(a). Balanced microalgae–microorganism nexus by using CDWW as growth medium.

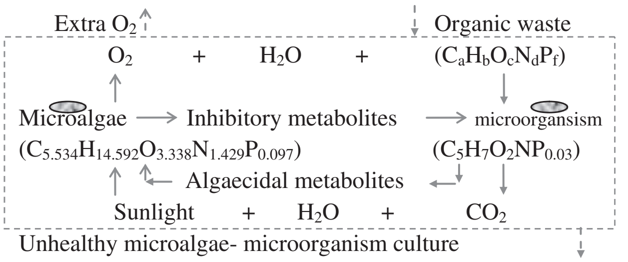


Fig. 1(b). Unbalanced microalgae–microorganism nexus by using CDWW as growth medium.

$CO_2$ is a core substrate for the microalgae growth. The microalgae–microorganism nexus is theoretically balanced when microalgae produce the same amount of molar $O_2$ which is required by the microorganisms. The microorganisms also generate the same amount of molar $CO_2$ which is required by the microalgae. In the theoretical balanced nexus, there is no extra $O_2$ or $CO_2$ evolved from the growth medium, since both of them are consumed during the growths (Fig. 1(a)).

The microalgae encourages the growth of microorganisms by providing extracellular compounds [7,8]. Similarly, the growth rate of microorganisms might enhance microalgae metabolism by providing growth-promoting factors [7–10] or by reducing dissolved $O_2$ concentration in the growth medium [7,8,11]. A high amount of extra $O_2$ or $CO_2$ in the growth medium might alter the chemical equilibrium, leading to an unbalanced relationship because the extra dissolved $O_2$ from microalgae could inhibit the aerobic microorganisms. The growth of aerobic microorganisms might reduce the amount of $CO_2$ supply required as substrate for microalgae (Fig. 1(b)).

A stoichiometric equation is essential to monitor microalgae–microorganism interaction. The stoichiometric equations with the biomass compositions of the microorganisms and microalgae are available in the literature as Eqs. (1a)-aeration, (1b)-photosynthesis, and (1c)-resultant [8].

$$C_7H_6O_3 + 0.396NO_3^- + 0.396H^+ + 4.08O_2 = 5.02CO_2 + 1.52H_2O + 1.98CH_{1.7}O_{0.4}N_{0.2} \quad (1a)$$

$$5.02CO_2 + 3.82H_2O + 0.75NO_3^- + 0.89H^+ + 0.047PO_4^{3-} = 5.02CH_{1.7}O_{0.4}N_{0.15}P_{0.009} + 7.15O_2 \quad (1b)$$

$$C_7H_6O_3 + 1.15NO_3^- + 0.047PO_4^{3-} + 2.31H_2O + 1.29H^+ = 3.07O_2 + 1.98CH_{1.7}O_{0.4}N_{0.2} + 5.02CH_{1.7}O_{0.4}N_{0.15}P_{0.009} \quad (1c)$$

The chemical formulas of essential chemicals in microorganisms ($CH_{1.7}O_{0.4}N_{0.2}$ [12]) and microalgae ($CH_{1.7}O_{0.4}N_{0.15}P_{0.009}$ [13]) were reported in the literature for the growth rate and substrate composition.

Microalgae are larger in size and typically grow slower when compared to heterotrophic microorganisms [14]. The specific growth rate of toluene-degrading *Pseudomonas* sp. is 0.4–0.8 $h^{-1}$ [15] while that of *Chlorella pyrenoidosa* in photosynthetic and heterotrophic culture is 0.082 and 0.038 $h^{-1}$, respectively [16]. The maximum specific growth rate of *Chlorella vulgaris* in photosynthetic, mixotrophic, and heterotrophic culture is 0.110, 0.098, and 0.198 $h^{-1}$, respectively [16]. In comparison with the microorganisms, it is therefore easier to inhibit microalgae growth by a higher level of substrate concentration. Microalgae can subsequently be used as sensors of acute toxicity [17,18]. Park et al. [19] recommended $C_{106}H_{181}O_{45}N_{16}P$ as the formula for the microalgae biomass typical composition and emphasized that the molar ratio of N/P in elemental composition of microalgae can diverge from about 4:1 to almost 40:1 based on the species and nutrient accessibility in the growth medium [19,20].

Since elemental composition of microalgae varies with species and nutrient availability from the growth medium, we hypothesize that the elemental composition of microalgae growing on CDWW may differ from the values reported in the literature due to the different species and the source of growth medium. The objectives of this study were to:

(1) determine elemental composition of microalgae that use nutrients from and grown in CDWW,
(2) examine the rate-limiting coefficients, and
(3) develop a generic stoichiometric equation for balancing reactions in the microalgae–microorganism nexus when CDWW is employed as a model organic substrate and growth medium.

Table 1
Referred and used characteristics of CDWW

| Referred from the literature | | | Used in calculation | |
|---|---|---|---|---|
| Parameters | mg/L | References | Parameters | mg/L |
| COD | 200–220 | * | COD | 210 |
| SS | 165–186 | * | TVS | 158 |
| Total N | 20–22 | * | Organic N | 20 |
| Total P | 2.6–4.0 | * | Organic P | 3 |
| pH, unitless | 7.6–8.4 | * | | |
| COD/C | 3.162 | ** | | |

Notes: *Referred from Aziz and Ng [21].
**Tchobanoglous and Burton [23], Tchobanoglous et al. [24].

Table 2
Design of experiments from Aziz and Ng [21] by using CDWW as growth medium

| Test | Nutrients added | |
|---|---|---|
| # | N% | P% |
| 1 | 0 | 0 |
| 2 | 15 | 0 |
| 3 | 30 | 0 |
| 4 | 45 | 0 |
| 5 | 60 | 0 |
| 6 | 75 | 0 |
| 7 | 0 | 0 |
| 8 | 0 | 15 |
| 9 | 0 | 30 |
| 10 | 0 | 45 |
| 11 | 0 | 60 |
| 12 | 0 | 75 |
| 13 | 0 | 0 |
| 14 | 15 | 15 |
| 15 | 30 | 30 |
| 16 | 45 | 45 |
| 17 | 60 | 60 |
| 18 | 75 | 75 |

## 2. Materials and methods

The stoichiometric equation for microalgae culture was developed based on experimental data of Aziz and Ng [21] (Table 1) by curve-fitting technique [2] using Microsoft Excel.

Eighteen sets of the laboratory data were divided into three groups based on nitrogen supplement, phosphorus supplement, and nitrogen and phosphorus supplements (Table 2). Based on the 18 different conditions, a generic stoichiometric equation was developed by using molecular elements of organic carbon (a), hydrogen (b), oxygen (c), nitrogen (d), and phosphorous (f) from the substrate CDWW.

Before establishing the stoichiometric equation, the elemental chemical formula of CDWW was formulated based on COD and molecular weight of substrate as shown in Eqs. (2) and (3) [22].

$$D = 8\ (4a + b - 2c - 3d + 5f) \tag{2}$$

$$TVS = 12a + b + 16c + 14d + 32f \tag{3}$$

$a = C/12$
$b = H/1$
$c = O/16$
$d = N/14$

$f = P/31$

where $D$: COD of organic matter in grams or measured COD; C: organic carbon in grams from TOC analysis; N: organic nitrogen in grams from Kjeldahl test; O: $O_2$ in grams; H: $H_2$ in grams; TVS: total volatile solid.

Microalgae and microorganism cell yields were found to be:

$$\begin{aligned}\text{Microalgae yield} = &\{(4a + 4v - r - 5y) + (b + 5v + 2w) \\ &- 2(c + 3v + 4w) - (3d + 3v + q) \\ &+ (5f + 5w - p)\}(1 + \mathrm{N}_{\mathrm{added},f})^4 \\ &\times (1 + \mathrm{P}_{\mathrm{added},f})^2 F/(\mathrm{MW}_{\mathrm{microalgae}})\end{aligned} \quad (4)$$

$$\begin{aligned}\text{Microorganism yield} = &\{(4a + 4v - r - 5.399x) \\ &+ (b + 5v + 2w) - 2(c + 3v + 4w) \\ &+ (3d + 3v - q) + (5f + 5w - p)\} \\ &\times (1 + \mathrm{N}_{\mathrm{added},f})^4 (1 + \mathrm{P}_{\mathrm{added},f})^2 \\ &\times F/(\mathrm{MW}_{\mathrm{cell}})\end{aligned} \quad (5)$$

$v$: molar coefficient for $HCO_3^-$ in generic stoichiometric equation in Table 4

$r$: molar coefficient for dissolved $CO_2$ in generic stoichiometric equation in Table 4

$y$: molar coefficient for $C_5H_7O_2NP_{0.03}$ in generic stoichiometric equation in Table 4

$w$: molar coefficient for $H_2PO_4$ in reactant of generic stoichiometric equation

$q$: molar coefficient for $NH_3$ in generic stoichiometric equation in Table 4

$p$: molar coefficient for $H_2PO_4$ in resultant of generic stoichiometric equation

$x$: molar coefficient for microalgae in generic stoichiometric equation in Table 4

MW: molecular weight.

## 3. Results and discussion

Chemical formulas for CDWW modeled from Aziz and Ng [21] and for microalgae grown in CDWW were found to be $C_{5.534}H_{14.592}O_{3.338}N_{1.429}P_{0.097}$ and $C_{5.399}H_{8.798}O_{2.173}NP_{0.241}$, respectively (Tables 3 and 4). The modeled stoichiometric coefficients in each reaction equation were summarized in Table 3 for each experiment of the 18 tests.

The fitted coefficients of correlation $R^2$ for microalgae and microorganisms yield were 0.908 in CDWW supplemented with nitrogen (Fig. 2(a)) and 0.878 in CDWW supplemented with phosphorus (Fig. 2(b)). The $R^2$ of 0.838 between measured and calculated coefficients in CDWW supplemented with nitrogen and phosphorus was observed (Fig. 2(c)), and the total $R^2$ of 0.849 was observed for CDWW with three different types of supplement (Fig. 2(d)). The strength of the linear relationship between the predicted and experimental data is high as shown on Fig. 2(a–d).

### 3.1. Reactions in microalgae bioreactor

The stoichiometric equation can be used to quantify the reactions in a reactor, optimize the microalgae yield, reduce the cost of added substances, increase the use of recycled waste materials to lower production cost, and minimize environmental pollution. Aziz and Ng [21] showed that the original contents of nitrogen and phosphorous in CDWW were not high enough to supplement the growth of microalgae and microorganisms. Their experiments showed that microalgae and microorganism yields increased when either nitrogen or phosphorous or both elements were added as supplements to CDWW in the amount of 15, 30, 45, and 60% increase in original value of nitrogen or phosphorus in CDWW. However, the yields decreased in the tests 6, 12, and 18 where 75% increase in the original values of either nitrogen or phosphorus or both in CDWW were added as supplements (Fig. 3). The tests with 75% supplemental content of nitrogen or phosphorus or both in the growth medium may have inhibited the growth of both microalgae and microorganisms. The decrease in yields might have been due to unbalanced substrate to nutrient ratio (C/N/P) and/or substrate to microalgae (microorganism).

The concentrations of nitrogen and phosphorus were 20–22 and 2.6–4.0 mg/L, respectively, in CDWW (Table 1), and the amounts of nitrogen and phosphorus $2.1 \times 10^{-5}$ g/g and $3.3 \times 10^{-6}$ g/g CDWW were in macro-level and micro-level, respectively, in one liter of growth medium for one liter equal to 998,200 mg (0.9982 kg) water in 20℃. However, such small amounts of nitrogen and phosphorus do not homogenously mix in one liter of growth medium. Therefore, it is necessary to add nutrients in the excess to compensate for the unused amount which resulted from inadequate mixing and precipitation from reactions with other substances. The quantities of unused nitrogen and phosphorus were defined as $\mathrm{N}_{\mathrm{unused}}$ and $\mathrm{P}_{\mathrm{unused}}$ to signify reactants not available to the microalgae and microorganism. From the modeling processes, the amounts $\mathrm{N}_{\mathrm{unused}}$ and $\mathrm{P}_{\mathrm{unused}}$ were found to be as 0.407 and 0.004 mol per mole of substrate, respectively.

Table 3
Modeled stoichiometric coefficients for each experimental run

| Test # | CDWW $C_{5.534}H_{14.592}O_{3.338}N_{1.429}P_{0.097}$ | Added $+O_2$ | Added $+CH_4$ | Added $+NH_4^+$ | Added $+HCO_3^-$ | Added $+H_2PO_4$ | Microalgae $=C_{5.399}H_{8.798}O_{2.173}NP_{0.241}$ | Microorganisms $+C_5H_7O_2NP_{0.03}$ | $+H_2O$ | $+CO_2$ | Residue $+NH_3$ | Residue $+H_2PO_4$ | Equation # |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| 1 | 1 | 4.747 | 0.207 | 0.000 | 0.000 | 0.000 | 0.156 | 0.257 | 4.547 | 3.612 | 1.016 | 0.052 | 5 |
| 2 | 1 | 3.689 | 0.605 | 0.214 | 0.214 | 0.000 | 0.276 | 0.479 | 4.803 | 2.467 | 0.888 | 0.016 | 6 |
| 3 | 1 | 3.740 | 1.003 | 0.429 | 0.429 | 0.000 | 0.315 | 0.580 | 5.511 | 2.367 | 0.963 | 0.004 | 7 |
| 4 | 1 | 4.423 | 1.400 | 0.643 | 0.643 | 0.000 | 0.310 | 0.608 | 6.478 | 2.864 | 1.154 | 0.004 | 8 |
| 5 | 1 | 5.093 | 1.798 | 0.857 | 0.857 | 0.000 | 0.306 | 0.638 | 7.441 | 3.349 | 1.342 | 0.004 | 9 |
| 6 | 1 | 5.747 | 2.196 | 1.071 | 1.071 | 0.000 | 0.302 | 0.671 | 8.396 | 3.818 | 1.528 | 0.004 | 10 |
| 7 | 1 | 4.747 | 0.207 | 0.000 | 0.000 | 0.000 | 0.156 | 0.257 | 4.547 | 3.612 | 1.016 | 0.052 | 11 |
| 8 | 1 | 3.960 | 0.207 | 0.000 | 0.000 | 0.015 | 0.211 | 0.347 | 4.226 | 2.869 | 0.871 | 0.050 | 12 |
| 9 | 1 | 3.023 | 0.207 | 0.000 | 0.000 | 0.029 | 0.276 | 0.453 | 3.842 | 1.983 | 0.699 | 0.046 | 13 |
| 10 | 1 | 1.914 | 0.207 | 0.000 | 0.000 | 0.044 | 0.354 | 0.579 | 3.389 | 0.936 | 0.496 | 0.038 | 14 |
| 11 | 1 | 1.219 | 0.207 | 0.000 | 0.000 | 0.058 | 0.388 | 0.635 | 2.774 | 0.475 | 0.407 | 0.043 | 15 |
| 12 | 1 | 1.216 | 0.207 | 0.000 | 0.000 | 0.073 | 0.388 | 0.635 | 2.774 | 0.473 | 0.407 | 0.057 | 16 |
| 13 | 1 | 4.747 | 0.207 | 0.000 | 0.000 | 0.000 | 0.156 | 0.257 | 4.547 | 3.612 | 1.016 | 0.052 | 17 |
| 14 | 1 | 2.347 | 0.605 | 0.214 | 0.214 | 0.015 | 0.366 | 0.635 | 4.253 | 1.198 | 0.642 | 0.004 | 18 |
| 15 | 1 | 2.231 | 1.002 | 0.429 | 0.429 | 0.029 | 0.414 | 0.759 | 4.894 | 0.937 | 0.685 | 0.004 | 19 |
| 16 | 1 | 2.119 | 1.400 | 0.643 | 0.643 | 0.044 | 0.456 | 0.887 | 5.539 | 0.679 | 0.729 | 0.004 | 20 |
| 17 | 1 | 1.921 | 1.798 | 0.857 | 0.857 | 0.058 | 0.500 | 1.031 | 6.150 | 0.335 | 0.755 | 0.004 | 21 |
| 18 | 1 | 2.087 | 2.196 | 1.071 | 1.071 | 0.073 | 0.517 | 1.135 | 6.910 | 0.335 | 0.848 | 0.011 | 22 |

Table 4
Coefficient formulas for generic stoichiometric equation

| CDWW | Added | Added | Added | Microalgae | Microorganisms | Residue | Residue | Equation |
|---|---|---|---|---|---|---|---|---|
| $C_aH_bO_cN_dP_f + u\ O_2$ | $+v\ NH_4^+$ | $+v\ HCO_3^-$ | $+w\ H_2PO_4$ | $= x\ C_{5.399}H_{8.798}O_{2.173}NP_{0.241}$ | $+y\ C_5H_7O_2NP_{0.03} + t\ H_2O + s\ CH_4 + r\ CO_2$ | $+q\ NH_3$ | $+p\ H_2PO_4$ | # |
| $x$ = mol of microalgae/mol CDWW | | | | | | | | |
| $x = \{(4a+4v-r-5y) + (b+5v+2w) - 2(c+3v+4w) - (3d+3v+q) + (5f+5w-p)\}\ (1+N_{added,\ f})^4\ (1+P_{added,\ f})^2\ F/(MW_{microalgae})$ | | | | | | | | 23 |
| $y$ = mol microorganism/mol CDWW | | | | | | | | |
| $y = \{(4a+4v-r-5.399x) + (b+5v+2w) - 2(c+3v+4w) + (3d+3v-q) + (5f+5w-p)\}\ (1+N_{added,\ f})^4\ (1+P_{added,\ f})^2\ F/(MW_{cell})$ | | | | | | | | 24 |
| $p$ = mol of phosphorous/mol CDWW = $f+w-0.241x-0.03y$ | | | | | | | | 25 |
| $q$ = mol of ammonia/mol CDWW = $d+v-x-y$ | | | | | | | | 26 |
| $r$ = mol of carbon dixoide/mol CDWW = $a+v-5.399x-5y-s$ | | | | | | | | 27 |
| $s$ = mol of methane/mol CDWW = $-(d-1)\ (a+v) + 0.357\ (b+5v+2w) - 0.714\ (c+3v+4w) - 1.071d + 2.143f + 0.664$ | | | | | | | | 28 |
| $t$ = mol of water/mol CDWW = $\{b+5v+2w-8.798x-7y-4s-3(d+v-x-y)-2(f+w-0.241x-0.03y)\}/2$ | | | | | | | | 29 |
| $u$ = mol of $O_2$/mol CDWW = $(2.173x+2y+t+2r+4p-4w-3v-c)/2$ | | | | | | | | 30 |
| $N_{added,\ f} = N_{added}\%/100$ | | | | | | | | 31 |
| $P_{added,\ f} = P_{added}\%/100$ | | | | | | | | 32 |
| $F$ = model fitting factor | | | | | | | | |

With reference to the stoichiometric parameters, the microalgae and microorganism yields either increased or decreased when CDWW was or was not supplemented with nitrogen or phosphorous are shown in Table 3. For example, in reactions of tests 1, 2, 7 to 10, and 13, the nitrogen and phosphorus were not limiting factors as the nitrogen and phosphorus were still available due to respective residual nitrogen > $N_{unused}$; phosphorus > $P_{unused}$ in the growth medium at the end of reactions in the right side of Eqs. (5), (6), (11)–(14), and (17), respectively. Similarly, in reaction Eqs. (7)–(10) and (18)–(21) of experiments numbers 3 to 6 and 14 to 17, phosphorus might have been the limiting factor because the residual phosphorus was equal to $P_{unused}$. Nitrogen might have been the limiting factor in the reactions of experiments 11 and 12, since the residual nitrogen was equal to $N_{unused}$ in the right side of Eqs. (15) and (16).

Figs. 2(a–c) and 3 showed the best correlation between calculated and experimental values from the Aziz and Ng data. All the 18 stoichiometric equations showed that phosphorus was the leading limiting factor followed by nitrogen as the processes consistently lead to $P_{min} < N_{min}$ (Figs. 4(a) and 4(b)).

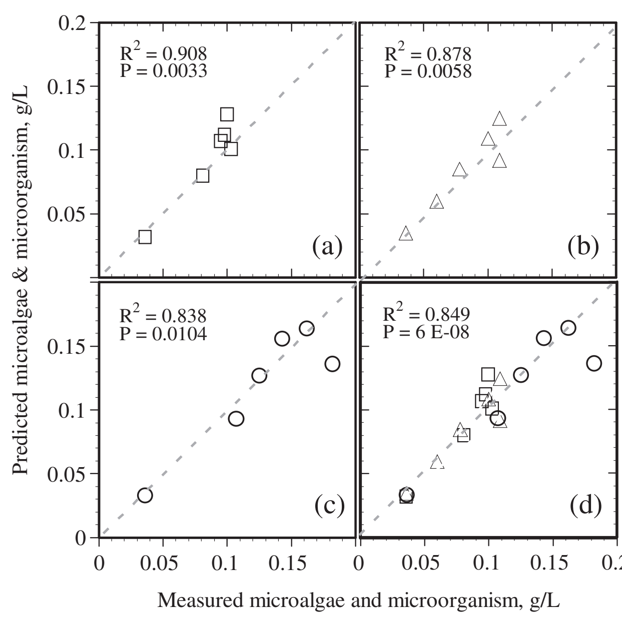


Fig. 2. Comparing microalgae and microorganisms between measured and calculated data. (a) CDWW supplemented with nitrogen; (b) CDWW supplemented with phosphorus; (c) CDWW supplemented with nitrogen and phosphorus; (d) all above three cases.

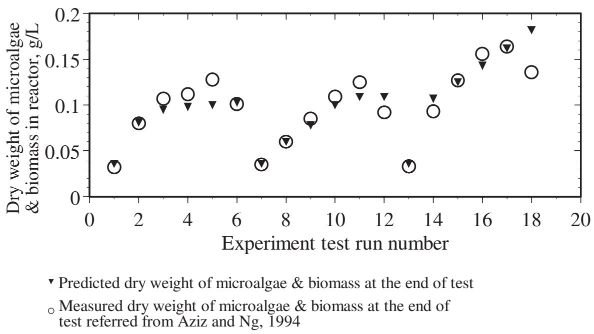


Fig. 3. Microalgae and microorganism yields by using CDWW as growth medium.
Tests 1, 7, 13: CDWW as growth medium without nitrogen and phosphorus supplements.
Tests 2–6: CDWW with the different amounts of nitrogen as supplement in growth medium. Tests 8–12: CDWW with the different amounts of phosphorus as supplement in growth medium.
Tests 14–18: CDWW with the different amounts of nitrogen and phosphorus as supplements.

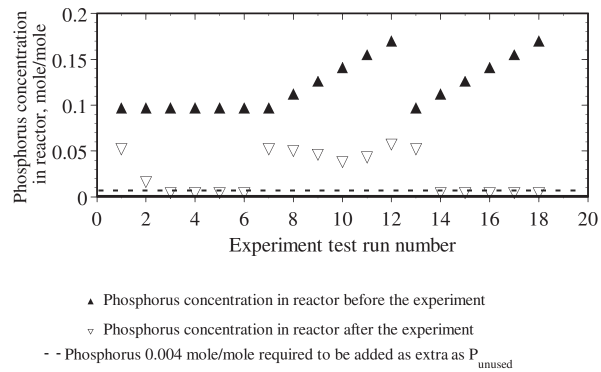


Fig. 4(b). Comparisons of phosphorus concentrations in reactor before and after the experiments.
Tests 1, 7, 13: CDWW as growth medium without nitrogen and phosphorus supplements.
Tests 2–6: CDWW with the different amounts of nitrogen as supplement in growth medium.
Tests 8–12: CDWW with the different amounts of phosphorus as supplement in growth medium.
Tests 14–18: CDWW with the different amounts of nitrogen and phosphorus as supplements.

### 3.2. Microalgae–microorganism nexus

Based on the values of stoichiometric coefficients determined from the experimental run by the model (Table 3), microalgae–microorganism nexus was established (Fig. 5). Fig. 5(a–e) show phosphorus, nitrogen, dissolved $CO_2$, microorganism, and microalgae concentrations at the end of the experiments for each test. In Table 3, the right side of all the reaction equations shows the resultants. Three types of carbon resultants were described as: microalgae carbon, microorganism carbon, and dissolved carbon dioxide. A high content of phosphorus, nitrogen, and dissolved carbon remained at the end of tests 1, 7, and 13 as shown by Eqs. (5), (11), and (17) of Table 3, Fig. 5(a–c). In tests 1, 7, and 13 microorganisms and microalgae growths were small as shown in Eqs. (5), (11), and (17) of Table 3, Fig. 5(d and e). Tests 1, 7, and 13 were presented an ineffective use of reactants into resultants, while tests 14–18 effectively used resources into products as smaller concentrations of phosphorus, nitrogen, and dissolved carbon remaining at the end of the tests as shown in Eqs. (18)–(22), Fig. 5(a–c). At the same time, the microorganism and microalgae growths were high as shown in Eqs. (18)–(22), Fig. 5(d and e). Unbalanced microalgae–microorganism nexus occurred in tests 4–6, where 50, 60, and 75% increase in original nitrogen was added into CDWW, respectively. In the tests 4–6, molar microalgae concentration decreased (Fig. 5(e)) but molar microorganism concentration increased (Fig. 5(d)) compared with the concentrations of test 3 probably due to the presence of algicidal metabolites (Fig. 1(b)). These results were a consequence of an excess of dissolved $CO_2$ concentration generated by the microorganisms (Eqs. (8)–(10) on Table 3), which might have inhibited microalgae growth [8]. Balanced microalgae–microorganism nexus were observed in test runs 8–12 and 14–18 (phosphorus was added in tests 8–12; nitrogen and phosphorus were added in test 14–18) where

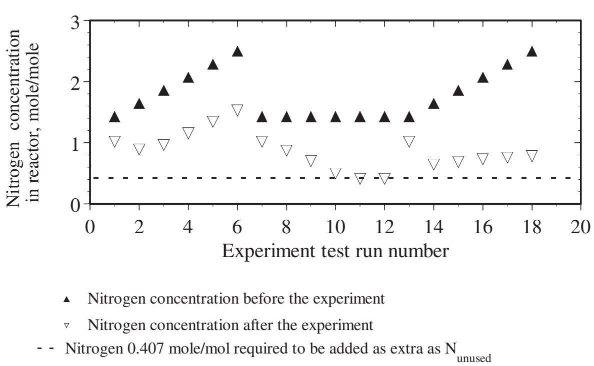


Fig. 4(a). Comparisons of nitrogen concentrations in reactor before and after the experiments.
Tests 1, 7, 13: CDWW as growth medium without nitrogen and phosphorus supplements.
Tests 2–6: CDWW with the different amounts of nitrogen as supplement in growth medium.
Tests 8–12: CDWW with the different amounts of phosphorus as supplement in growth medium.
Tests 14–18: CDWW with the different amounts of nitrogen and phosphorus as supplements.

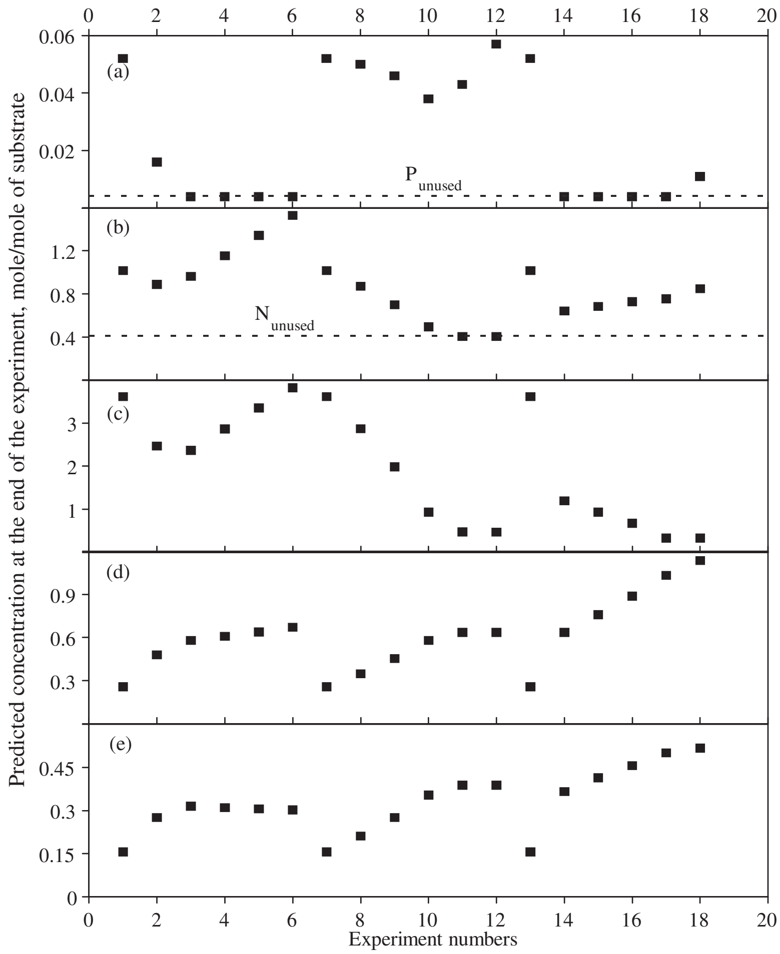


Fig. 5. Microalgae–microorganism nexus. (a) Phosphorus, (b) nitrogen, (c) dissolved carbon dioxide, (d) microorganism, and (e) microalgae concentrations at the end of the experiments.
Tests 1, 7, 13: CDWW as growth medium without nitrogen and phosphorus supplements.
Tests 2–6: CDWW with the different amounts of nitrogen as supplement in growth medium.
Tests 8–12: CDWW with the different amounts of phosphorus as supplement in growth medium.
Tests 14–18: CDWW with the different amounts of nitrogen and phosphorus as supplements.

both molar concentrations of microalgae and microorganisms increased (Fig. 5(d and e)), while the molar concentrations of $CO_2$ decreased (Fig. 5(c)). However, the molar concentrations of microalgae and microorganisms for the test 11 (in which 60% P was added) and 12 (where 75% of phosphorus was added) were the same due to the nitrogen limitation in test 12 (Fig. 5(b)). The optimal C/N/P molar ratio was predicted from test 17 (Eq. (21) of Table 3) in which the highest dry weight of microalgae and microorganism yields were observed ([21] and Fig. 3). The optimal nutritional molar ratio in the medium for microalgae and microorganism growth was established to be C/N/P = 53:15:1.

## 4. Conclusion

A generic stoichiometric equation for microalgae growth by using CDWW was developed based on 18 sets of experimental data from Aziz and Ng. Microalgae–microorganism nexus was established by using the developed stoichiometric coefficients. The study showed that nitrogen and phosphorus should be added to CDWW in order to acquire a balanced microalgae–microorganism nexus. To generate a high microalgae and microorganisms yield from a balanced microalgae–microorganism nexus and the effective recycling of waste resource, an optimal nutritional molar C/N/P ratio was proposed to be 53:15:1. The developed stoichiometric equation and optimal molar ratio of carbon, nitrogen, and phosphorus could be used to design laboratory, pilot, and large-scale microalgae production using cheap organic waste substrate thus cutting operational cost, reduce demand for physical labor, and minimize time constraints.

## Acknowledgments

This project was supported in part by the Office of Naval Research (ONR) from the USA (Contract # N00014-08-1-0304) by Tier I: Reclamation Cooperative Agreement Program through Bureau of Reclamation GR0002841-GHASSEMI BOR COOP by the NSF Engineering Research Center: RenuWIT, and by the Ed & Harold Foreman Endowed Chair. We also thank anonymous reviewers and the editor from DWT for their constructive professional comments and advice.

## References


[1] M.T. Myint, N. Nirmalakhandan, Evaluation of first-order, second-order, and surface-limiting reactions in anaerobic hydrolysis of cattle manure, Environ. Eng. Sci. 23 (2006) 970–980.

[2] M.T. Myint, N. Nirmalakhandan, R.E. Speece, Anaerobic fermentation of cattle manure: Modeling of hydrolysis and acidogenesis, Water Res. 41 (2007) 323–332.

[3] F.M. Fisher, A.T. Huber-Lee, The value of water: Optimizing models for sustainable management, infrastructure planning, and conflict resolution, Desalin. Water Treat. 31 (2011) 1–23.

[4] A.A. Konan, D. Guiral, Algal biomass available in artificial habitats for a tropical brackish water fish: *Sarotherodon melanotheron*: Fifth International Conference on Artificial Habitats for Fisheries, Long Beach, CA, USA, November 3–7, 1991.

[5] L. Batan, J. Quinn, B. Willson, T. Bradley, Net energy and greenhouse gas emission evaluation of biodiesel derived from microalgae, Environ. Sci. Technol. 44 (2010) 7975–7980.

[6] M.T. Myint, A. Ghassemi, N. Nirmalakhandan, Review of nondestructed anaerobic digested sludge to reinstate concentrate from electrodialysis reversal desalination in bioenergy production, Environ. Eng. Sci. 27 (2010) 811–823.

[7] G.M. Wolfaardt, J.R. Lawrence, R.D. Robarts, D.E. Caldwell, The role of interactions, sessile growth, and nutrient amendments on the degradative efficiency of a microbial consortium, Can. J. Microbiol. 40 (1994) 331–340.

[8] R. Muñoz, B. Guieysse, Algal–bacterial processes for the treatment of hazardous contaminants: A review, Water Res. 40 (2006) 2799–2815.

[9] M. Fukami, H. Ohbayashi, Y. Yoshioka, M. Kuroishi, S. Yamazaki, S. Toda, A zinc tolerant chlorotic mutant strain of *Euglena-Gracilis-Z* induced by zinc (Microbiology & fermentation industry), Agric. Biol. Chem., Tokyo 52 (1988) 601–604.

[10] L.E. Gonzalez, Y. Bashan, Increased growth of the microalga *Chlorella vulgaris* when coimmobilized and cocultured in alginate beads with the plant-growth-promoting bacterium *Azospirillum brasilense*, Appl. Environ. Microbiol. 66 (2000) 1527–1531.

[11] J.L. Mouget, A. Dakhama, M.C. Lavoie, J. De la Noüe, Algal growth enhancement by bacteria: Is consumption of photosynthetic oxygen involved? FEMS Microbiol. Ecol. 18 (1995) 35–43.

[12] B. Atkinson, F. Mavituna, Stoichiometric aspects of microbial metabolism, in: B. Atkinson, F. Mavituna (Eds.), Biochemical Engineering and Biotechnology Handbook, The Nature Press, New York, NY, 1983, pp. 114–203.

[13] W.J. Oswald, Micro-algae and waste-water treatment, in: M. B.L. Borowitzka (Ed.), Micro-Algal Biotechnology, Cambridge University Press, Cambridge, 1992, pp. 305–328.

[14] T. Fenchel, Intrinsic rate of natural increase: The relationship with body size, Oecologia 14 (1974) 317–326.

[15] K.F. Reardon, D.C. Mosteller, J.D.B. Rogers, Biodegradation kinetics of benzene, toluene, and phenol as single and mixed substrates for *Pseudomonas putida* F1, Biotechnol. Bioeng. 69 (2000) 385–400.

[16] Y.K. Lee, Microalgal mass culture systems and methods: Their limitation and potential, J. Appl. Phycol. 13 (2001) 307–315.

[17] OECD/OCDE 201, OECD Guidelines for the Testing of Chemicals: Freshwater Alga and Cyanobacteria, Growth Inhibition Test. The Organization for Economic Cooperation and Development. Available from: <http://www.oecd-ilibrary.orgdocserver/download/9720101e.pdf?expires=1365176088&id=id&accname=guest&checksum=67BBAFBB80C5EDA41C35CA2C110E0FE5> April 05, 2013.

[18] C.Y. Chen, J.H. Lin, Toxicity of chlorophenols to *Pseudokirchneriella subcapitata* under air-tight test environment, Chemosphere 62 (2006) 503–509.

[19] J.B.K. Park, R.J. Craggs, A.N. Shilton, Wastewater treatment high rate algal ponds for biofuel production, Bioresour. Technol. 102 (2011) 35–42.

[20] R.J. Craggs, S. Heubeck, T.J. Lundquist, J.R. Benemann, Algae biofuel from wastewater treatment high rate algal ponds, Water Sci. Technol. 63 (2011) 660–665.

[21] M.A. Aziz, W.J. Ng, Algal biotechnology, in: P.S. Moi, L.Y. Kun, M.A. Borowitzka, B.A. Whitton (Eds.), The Asia-Pacific Region Institute of Advanced Studies, University of Malaya, Kuala Lumpur, 1994, pp. 284–290.

[22] R.E. Speece, Anaerobic Biotechnology for Industrial Wastewaters, Archae Press, Nashville, TN, 1996.

[23] G. Tchobanoglous, F.L. Burton, Wastewater Engineering Treatment, Disposal, and Reuse, McGraw-Hill, Boston, MA, 1991.

[24] G. Tchobanoglous, F.L. Burton, H.D. Stensel, Wastewater Engineering Treatment and Reuse, Tata McGraw-Hill, New Delhi, 2003.