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Effects of Inorganic Ions and Glucose on Hydrogen Production by Indigenous Microbes in Sewage Sludge

Jaruwan Wongtanet [a] and Benjaphorn Prapagdee [a]

[a] *Laboratory of Environmental Biotechnology, Faculty of Environment and Resource Studies, Mahidol University, Salaya, Nakhonpathom 73170, Thailand*

Abstract

The effects of inorganic ions (Cl^-, K^+, Na^+ and Mg^{2+}) and glucose concentrations on hydrogen production using sewage sludge microbes were studied. The experiments were investigated in the batch experiment by the heat mixed microorganisms at pH 5.5 and 35 ^oC. Cumulative hydrogen production increased with time for each ion and concentration to a maximum after 5-6 days. Hydrogen production decreased directly with inorganic ion concentrations. Generally, cumulative hydrogen production for a given time was highest in the absence of each ion except for Na^+. Glucose concentration has affected on hydrogen and volatile fatty acid (VFA) production. The optimal loading concentration of glucose is about 20 g/l for maximum hydrogen production.

Keywords*:* batch experiment; hydrogen production; ionic concentration; organic loading

1. Introduction

Combustion of fossil fuels and natural gases provide about 80% of global energy; however, their end products, collectively known as greenhouse gases, are the principal causes of unfavorable elevations in atmospheric temperature. Hydrogen is an alternative energy source without this concern (Das and Veziroglu, 2001) and with the potential to be available at low cost (Mizuno *et al*., 2000). Municipal, agricultural and industrial waste materials containing high concentrations of organics may provide for the production of hydrogen using anaerobic microbial flora without contributing to green house gases (Das and Veziroglu, 2001; Montgomery, 2004). This coupling of hydrogen production to waste utilization may simultaneously provide economic and environmental benefits (Oh *et al*, 2003). Some inorganic ions in sludge and organic wastes can directly or indirectly inhibit microorganism activities including hydrogen production (Lee *et al*., 2001; Lin and Lay, 2005). Hydrogen is a key intermediate in the overall anaerobic oxidation-reduction reactions involved in the mineralization of organic matter, and the concentration of hydrogen plays a central role in controlling the proportions of the various products generated during the fermentation (Nielsen *et al*., 2001). Hence, using substrates contained plentiful of ions may likely inhibit culture fermentation.

The purposes of this study were to understand the effects of four inorganic ions, Na^+, Cl^-, K^+, and Mg^{2+}, and glucose concentrations on hydrogen and volatile fatty acid (VFA) production using sewage sludge microbial.

2. Materials and methods

Seed sludge

Seed sludge was obtained from an anaerobic digester in a municipal wastewater treatment plant in Bangkok, Thailand. Prior to use, seed sludge was heated at 102 ^oC for 20 min to inhibit methane-producing bacteria activity and to enrich the hydrogen-producing bacteria (Van Ginkel *et al*., 2001). The characteristics of seed sludge were COD 11,900 mg/l, suspended solid (SS) 8,100 mg/l and volatile suspended solid (VSS) 5,400 mg/l.

Experimental conditions

Five series of batch experiments were conducted in serum vials each with a volume of 160 ml. The vials were initially purged with argon followed by the addition of 20 ml of seed sludge and 120 ml of medium. In all experiments, the bacteria obtained from sludge treated as describe aboved were used as seed.

Series 1 examined the effects of glucose concentrations on hydrogen production.

The chemical medium formulated to serve as the hydrogen source was prepared from glucose (5, 10, 15, 20, 25, 30 and 40 g/l) containing fixed and sufficient inorganic concentrations for optimum microbial growth such as : NH_4HCO_3 5,240 mg/l, $NaHCO_3$ 6,720 mg/l, K_2HPO_4 125 mg/l, $MgCl_2 \cdot H_2O$ 100 mg/l, $MnSO_4 \cdot$

H_2O 15 mg/l, $CuSO_4 \cdot 7H_2O$ 5 mg/l, $CoCl_2 \cdot 5H_2O$ 0.125 mg/l, and $FeSO_4 \cdot 7H_2O$ 25 mg/l (Lay *et al*., 1999).

Series 2-4 investigated the effects of different ionic formulas and concentrations such as Na^+($NaHCO_3$), Cl^-(NaCl), K^+(K_2CO_3) and Mg^{2+}($Mg(NO_3)_2 \cdot 6H_2O$) at 1,000, 3,000, 5,000, 10,000, 15,000 and 20,000 mg/l, respectively in a solution of glucose fixed at 20 g/l.

The pH was adjusted to about 5.5 by addition of HCl or NaOH. Then, each vial was flushed with Argon for 5 min to assure anaerobic conditions, capped, and placed in an incubator shaker (35 °C; 120 rpm) in the dark for 240 h. Gas production was measured by the plunger displacement method with appropriately sized wetted glass syringes, ranging from 10 to100 ml (Owen *et al*., 1979).

Analytical methods

Gas composition was determined by a gas chromatography with an instrument equipped with a thermal conductivity detector (TCD) and a 1.5 m stainless-steel column packed with 5 Å molecular sieve (Aligent Technologies, Model 6890N). Argon was used as the carrier gas at a flow rate of 30 ml/min. The operational temperatures of the injector port, oven and detector were 100, 50 and 200 °C, respectively. Liquid samples were collected by syringe after 240 h of incubation period for volatile fatty acid (VFA) analysis. VFA detection was analyzed with a gas chromatography using a flame ionization detector (FID) and a 30 m x 0.25 mm x 0.25 µm HP-INNOWax Polyethylene Glycol Innowax column. Temperatures of injection port, oven and detector were 220, 110, and 240 °C, respectively. Argon was again the carrier gas at 30 ml/min.

Kinetic parameters

The modified Gompertz equation (Eq. 1) was used to fit the cumulative hydrogen production in batch experiments to obtain the hydrogen production potential *P*, the hydrogen production rate *R*, and lag phase λ.

$$H = P \exp\left\{-\exp\left[\frac{R_m . e}{p}(\lambda - t) + 1\right]\right\} \quad (1)$$

where, *H* is the cumulative hydrogen production (ml), λ the lag-phase time (h), *P* the hydrogen production potential (ml), R_m the maximum hydrogen production rate (ml/h), *t* the incubation time (h), *e* the exp(1) = 2.718. Parameters (*P*, R_m and λ) were estimated using the solver function in Microsoft Excel version.

3. Results and discussion

3.1. Effect of inorganic ion concentrations on hydrogen production

Individual batch experiments were followed until hydrogen production ceased. Product analysis shows that the anaerobic fermentation produced a biogas that contains only hydrogen and carbon dioxide, without detectable methane and sulfurated hydrogen, suggesting that no methane and sulfate-reducing bacteria are active in the cultures used in this study.

Highest cumulative hydrogen production, 136 ml H_2, was at the sodium concentration of 1,000 mg Na^+ as $NaHCO_3$ mg/l [Fig. 1(a) and Table 1]. Hydrogen production decreased at concentrations of 3,000, 5,000 and 10,000 mg Na^+ as $NaHCO_3$ mg/l, respectively. Hydrogen production did not occur at sodium concentration higher than 10,000 mg Na^+ as $NaHCO_3$ mg/l.

Cumulative hydrogen production decreased with the increasing of chloride concentration [Fig. 1(b) and Table 1]. The highest cumulative hydrogen production was about 123 ml in the control.

The modified Gompertz equation (Eq. 1) has been applied to describe the cumulative hydrogen production process obtained from the batch experiments (Lee *et al*., 2001; Liu and Shen, 2004; Lin and Lay, 2004). In order to evaluate the effects of sodium and chloride concentrations on cumulative hydrogen production, experimental data were fitted to Eq. (1). The estimated values of the hydrogen production potential (P), the maximum hydrogen production rate (Rm), the lag-phase time (λ) and the correlation coefficient (R^2) were calculated and summarized in Table 2. Results of the calculation clearly indicated that the hydrogen production potential and rate decreased with the increasing of the Na^+ and Cl^- ion concentrations (Table 2). Moreover, the lag phase time was increased till 31 h. All correlation coefficients of non-linear analysis by Eq. (1) exceeded 0.99, suggesting that the modified Gompertz model was able to adequately describe the hydrogen production from glucose by mixed cultures in medium contained sodium and chloride concentrations.

The effect of magnesium concentration on hydrogen production is shown in Fig. 1(c). Highest hydrogen production was observed in the control sample approximately 164 ml. The hydrogen production was slightly decreased when magnesium concentration increased from 1,000 mg Mg^{2+} as $Mg(NO_3)_2 \cdot 6H_2O$ mg/l and was not produced while the magnesium concentration was higher than 5,000 mg Mg^{2+} as $Mg(NO_3)_2 \cdot 6H_2O$ mg/l.

Similar results were observed in the potassium batch experiment. Highest hydrogen production was found in the control sample of 110 ml [Fig. 1(d) and Table 1]. The hydrogen production was affected by the increasing potassium concentration.

It was concluded that cumulative hydrogen production increased with time for each ion and concentration to a maximum after 5-6 days [Fig. 1(a-d)]. Hydrogen production directly decreased with inorganic ion concentrations. Generally,

Table 1. Summary of the effect of ionic concentrations on the fermentation products

Ionic formula	Ionic concentration (mg/l)	Cumulative hydrogen production (ml)	Products (mg/l)		B/A
			HAc	HBu	
Na^+	0	117.33	9056.05	12155.03	1.34
($NaHCO_3$)	1000	136.02	10628.94	15413.78	1.45
	3000	115.82	8890.39	10989.37	1.24
	5000	105.14	8440.58	10628.94	1.26
Cl^-	0	122.7	9369.25	14286.08	1.52
(NaCl)	1000	80.22	7757.71	9056.05	1.17
	3000	67.97	6661.17	7357.75	1.10
	5000	23.04	4565.30	3030.2	0.66
Mg^{2+}	0	164.30	12155.03	18757.69	1.54
($Mg(NO_3)_2 \cdot 6H_2O$)	1000	124.78	9450.25	14450.08	1.53
	3000	96.53	8609.68	10254.31	1.19
	5000	82.01	7915.75	9369.25	1.18
K^+	0	113.68	8740.58	10669.37	1.22
(K_2CO_3)	1000	110.43	8838.39	10528.94	1.19
	3000	84.69	7715.75	9269.25	1.20
	5000	66.14	6446.86	7157.75	1.11

cumulative hydrogen production for a given time was highest in the absence of each ion except for Na^+ [Fig. 1(a)].

3.2. *Effect of inorganic ion concentrations on VFA production*

The formation of hydrogen is accompanied with the VFA or solvent production during an anaerobic fermentative process. The major VFA produced in the experiments were acetate (HAc) and butyrate (HBu). Therefore, the formation of these metabolites during hydrogen fermentation is often a crucial signal for assessing the efficiency of hydrogen-producing cultures and is useful indicators for monitoring hydrogen production (Dinopoulou *et al*., 1998; Cha and Noike, 1997; Lee *et al*., 1999).

Results of this investigation revealed that HAc and HBu decreased with increment of all ionic concentrations, except sodium test (Table 1). At 1,000 mg Na^+ as $NaHCO_3$ mg/l, HAc and HBu were increased; however decreased at the higher

Table 2. Kinetic parameters on hydrogen fermentation calculated from non-linear regression of Eq. (1)

Ionic formula	Ionic concentration (mg/l)	P (ml H_2)	R_m (ml/h)	λ (h)	R^2
Na^+	0	117.33	2.12	0	0.990563
($NaHCO_3$)	1000	136.06	1.96	6	0.990068
	3000	115.82	1.47	8	0.990563
	5000	105.14	1.31	11	0.990314
Cl^-	0	122.7	2.18	0	0.997139
(NaCl)	1000	80.22	1.15	25	0.997280
	3000	67.97	0.72	31	0.993515
	5000	23.04	0.29	31	0.993749

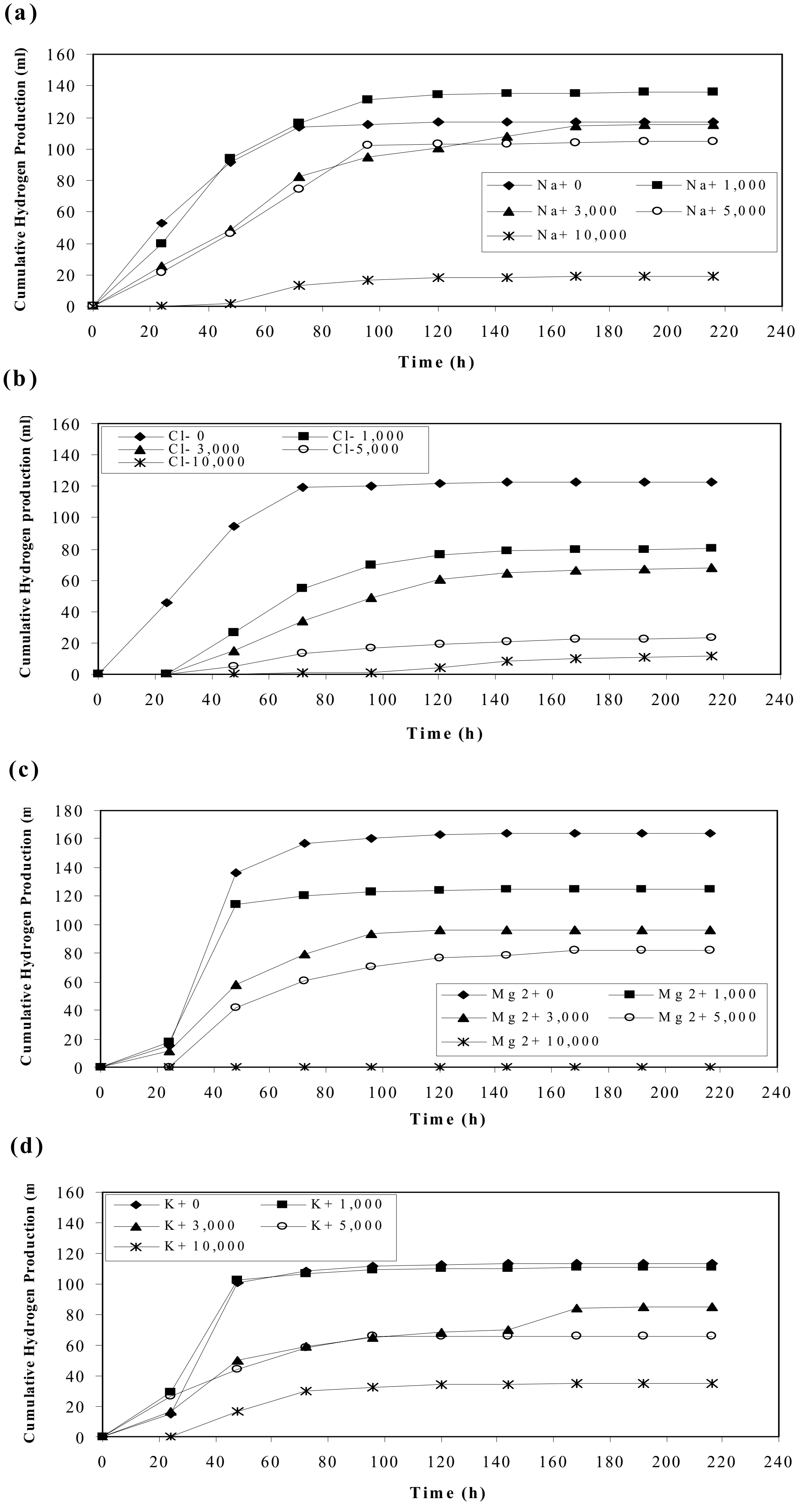

Figure 1. Hydrogen gas produced in batch tests with different ionic formulas and concentrations. (a) 0-10,000 mg Na^+ as $NaHCO_3$ mg/l (b) 0-10,000 mg Cl^- as NaCl mg/l (c) 0-10,000 mg Mg^{2+} as $Mg(NO_3)_2.6H_2O$ mg/l, and (d) 0-10,000 mg K^+ as K_2CO_3 mg/l.

Table 3. Volatile fatty acid (VFA) production at different glucose concentrations

Glucose concentration (g/l)	Volatile fatty acid (VFA) production (mg/l)		
	Acetate	Butyrate	Total
5	2571.01	239.41	2810.42
10	3284.78	7024.85	10309.63
15	3818.63	7566.30	11384.93
20	4092.58	10311.14	14403.72
25	2897.27	6135.23	9032.50
30	2347.51	492.82	2840.33
40	2516.47	232.11	2748.58

concentration than 1,000 mg Na^+ as $NaHCO_3$ mg/l. This occurrence showed that sodium concentration (0-1,000 mg Na^+ as $NaHCO_3$ mg/l) may be useful for hydrogen production. Meanwhile, the available hydrogen from glucose degradation during hydrogen fermentation has been determined using HBu/HAc (B/A) ratio (Nandi and Sengupta, 1995; Ueno *et al.*, 1995) and has frequently been used as the indicator for evaluating effectiveness of hydrogen production (White 1995; Annous *et al.*, 1996). For the chloride concentration (0-5,000 mg Cl^- as NaCl mg/l), the ratios of B/A are 0.66-1.52, which are decreased with increasing chloride concentration. The values of B/A are 1.11-1.22 and 1.18-1.53 in case of potassium and magnesium concentrations, respectively. For sodium concentration, the values of B/A are 1.24-1.45. The above results suggest that the formation of HBu in this study seems to favor hydrogen production. Nevertheless, the optimal B/A ratios for hydrogen production vary with the differences of anaerobic fermentative cultures and substrate used. For instance, using sucrose as the carbon substrate and study the effect of the iron concentration on the fermentative products, the B/A ratios for the hydrogen-producing cultures were 2-3 and 3-4 (Khanal *et al.*, 2004; Zhang *et al.*, 2005). The overall B/A ratios in this study (0.66-1.54) were lower than previous studies. This probably dues to different carbon sources and ionic concentrations used in the study.

3.3. Effect of glucose concentration on hydrogen production

The cumulative hydrogen production from various glucose concentrations is shown in Fig. 2. It shows that the organic loading of glucose has an effect on hydrogen production. At the concentration of 20 g glucose/l, the cumulative hydrogen production was

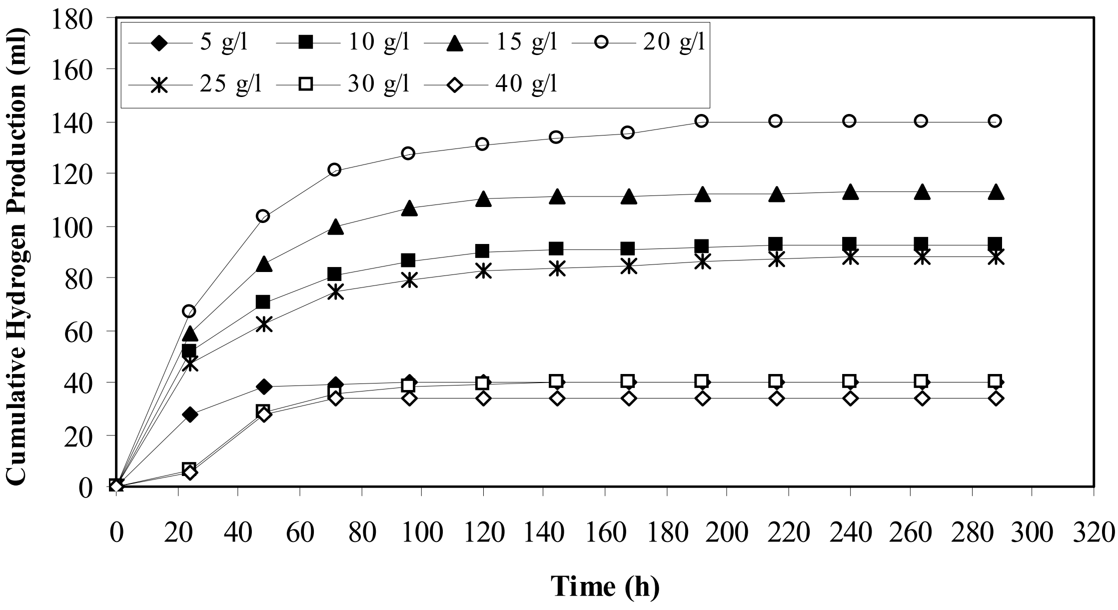

Figure 2. Effect of glucose concentrations on hydrogen production.

produced at the highest amount of 140 ml. Subsequently, it was significantly decreased while the loading was higher than 20 g glucose/l. This is probably due to the shock loading of glucose concentration in the process and may be affected on the hydrogen-producing bacteria. Hence, limitation of organic loading of substrate should be one factor of concern. From above results, it may be usefully applied in design of continuous process which regards to hydraulic retention time (HRT) of system.

3.4. Effect of glucose concentration on VFA production

From the study, variations in glucose concentration, total VFA productions were about 2,810-14,403 mg/l. However, the major VFAs detected in the process were acetate and butyrate. The former was in the range of 2,516- 4,092 mg/l and the latter was 232-10, 311 mg/l (Table 3). The high butyrate concentration revealed that the reaction may be a butyrate fermentation type. *Clostridium* species was considered to be the dominant organisms in this study because these organisms are responsible for butyrate fermentation (Yokoi *et al.*, 1997; Dinopoulou *et al.*, 1998). Furthermore, butyrate concentration increased maximumly at glucose concentration of 20 g/l and decreased when it surpassed 20 g/l. It can be concluded that the glucose concentration has affected on hydrogen and VFA production. The optimal loading concentration of glucose is about 20 g/l for maximum hydrogen peoduction.

4. Conclusion

Results of this study indicated that the inorganic ion and glucose concentrations have the effects on the hydrogen and VFA production. This study revealed that the existence of chloride, potassium and magnesium concentrations had obviously inhibited the hydrogen-producing bacteria when their concentrations increased in the range of 0 to 5,000 mg/l. Among these experiments, chloride was the most harmful ion on hydrogen production. Furthermore, results of the experiment showed that the sodium concentration at 1,000 mg Na^+ as $NaHCO_3$ mg/l could promote the metabolism of butyrate and hydrogen production. However, the effect of low sodium concentrations (0-1,000 mg Na^+ as $NaHCO_3$ mg/l) on the hydrogen-producing bacteria should be under in-depth investigation. On the other hand, glucose concentration at 20 g/l shows a greater enhancement of hydrogen and VFA production.

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Correspondence to
Dr. Jaruwan Wongtanet
Faculty of Environment and Resource Studies,
Mahidol University, Salaya, Nakhonpathom 73170,
Thailand
Email: enjwt@mahidol.ac.th
Fax: 662 441 9509-10;
Tel: 662 441 5000 ext. 1318