

Potential of Thai Soil on the Anaerobic Treatment of Urban Wastewater in the Alternate Flooding and Drying of the Soil with Plant System

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Abstract

To evaluate potential treatment of organic waste (OW) dominant pollutions in the urban wastewater by Thai soils, a primary and a main anaerobic incubation experiments have been carried out. First probation, excess glucose solution (1500 mg/l) without (0) and with (+3750 mg/l of 16-20-0 fertilizer) in a 5 x 2 factorial in CRD, having 5 flooding periods: 0, 1, 3, 5 and 7 days and 2 fertilization: 0 and + replicated 3 times, in a Tropequepts (Rb series) at 1:2 of soil:solution ratio, has been carried out. This trial confirmed, LERD's discovery that anaerobic treatment of the OW was very effective reaching the treatment potential at the 5 days period at 2730 ppm from the 3000 ppm load used. Next demonstration, the soil and other 16 ones in 7 of the 9 Thai soil Orders which were Alfisols (4), Inceptisols (3), Mollisols (3), Ultisols (3), Vertisols (2), Spodosols (1), and Oxisols (1) were investigated. Anaerobic incubation at 1:2 soil:solution ratio of the fertilized glucose solution at 5 days period in the CRD experiment of the 17 soils with 3 replications that was highly significant difference ($P < 0.01$). Soil properties had correlated with glucose treatment by Pearson correlation, were related in linear equation which can forecasted potential of wastewater treatment because of many soil properties were encourage anaerobe activities which are nutrients, enzymes and electron accepters.

Keywords: ferric iron, ferrus iron, alternate flooding and drying soil, wastewater treatment

1. Introduction

Contamination of untreated urban wastewater into any surface water resources, at the load higher than its carrying capacity are detrimental to all aerobic lifes, since the sludge always composes of such microbial nutritious substances as carbohydrates and proteins (Hammer & Bastain, 1989), dissolve oxygen (DO) in the contaminated river will be rapidly used up by all aerobic decomposers. Regulations on organic wastewater disposal in developed countries have been, therefore, enforced (Focazio et al., 2008; NDWRCDP, 2005; Taibe & Droste, 2004). Thailand has also realized this problem and has set her own wastewater disposal standard to be not higher than 20 mgO₂/l BOD (Pollution Control Department, 2006).

Such efficient wastewater treatment system as Activated Sludge (AS) and Upflow Anaerobic Sludge Blanket (UASB) in food and beverage industries used worldwide are too expensive to not only in the construction but also the operation and maintenance costs (Chong et al., 2012; Friedler & Pisanty, 2006; Sacho & Garrido, 2009; Wei et al., 2001). Alternate Flooding and Drying of Soil with Plant System (AFDSP) for community wastewater developed by LERD in Petchaburi Province, Thailand that system can treat wastewater at rural area before drain it to water resources. The main principle of this AFDSP is to utilize the indigenous soil facultative anaerobes to decompose, the wastewater organic carbon (OC) submerged into completely dry paddy soil for 5 days, anaerobically, and aerobically after disposal the treated water and 2 days of sun drying of the system. Cycles of this 7 days cycle each efficiently treated the sludge of about 100 mgO₂/l BOD to only about 10 mgO₂/l BOD

leachate at the first cycle; with and without plants, tolerated to 5 days flooding tested, gave no significant different efficiency. Performance of this system can last longer than 2 months as evidenced by three theses (Boonanake, 1996; Tangjaikrongbun, 1997; Tantanararit, 1995). Though horizontal mechanism of sludge treatment by the plants cultivated were not as clear as that of the vertical one of the soil facultative decomposers, the plants help remove mineralized organic N (ON) and the $\text{H}_2\text{PO}_4\text{-P}$ released from the Fe(III)-P reduction as electron acceptor (Tasutsuki & Ponnampereuma, 1987; Truu et al., 2009; Unger et al., 2009). Ecological benefits of the plants cultivated over the bare soil system were the additional superiority. Another advantage of this inexpensive system is that after the Fe(III) compounds, which are the most abundant electron acceptors, have been used up to be Fe(II) compounds or the treatment potential is reached, this system can be completely reactivated by sun drying, grinding and redrying to assure complete dryness (Lovley, 1991, 1997).

Understanding on ammonification and the release of other plant nutrients in paddy soil during flooding period using soil organic matter and crop residue as electron donor and such electron acceptors available in the soil as nitrate, manganese dioxide (MnIV-Mn) and oxide or hydroxide of ferric compounds (FeIII-Fe) by obligate anaerobes in the anaerobic decomposition process have already been realized (Ponnampereuma, 1965, 1972).

Prabuddham (1975) reviewed and elucidated association of such micronutrients as Mn, Cu, Zn and Co, such possible pollutant as Cr, Ni and Pb, to the fractions of Fe(III) in paddy soils of Thailand and South Vietnam. Release of these microelements during reduction of the active Fe (FeIII) as well as the active Mn(MnVI) mentioned is, therefore, possible and ecologically important. Decomposers involved, however, have just intensively investigated recently (Lovley & Phillips, 1996). Minimization of methane gas in the soil, high in Fe(III) has also been reported (Huang et al., 2009; Kjaergaard et al., 2012; Zhang et al., 2010).

Utilization of this anaerobic process in submerged soil on organic wastewater treatment has been overlooked until the recently report (Tantanararit, 1995). In the report modification of the continuous anaerobic condition, which is manipulated by obligate anaerobes in the paddy soil, to be alternate flooding and drying so that much more active facultative aerobes can play the important role. The 10 days cycle of 7 days flooding and 3 days sun drying after disposal, successfully treated the sewage of about 80 mgO_2/l to about 10 mgO_2/l BOD since the first cycle throughout this 90 days period of investigation. Such salt tolerant plants as *Cyperus corymbosus*, *Letoschlosa fusca*, *Typha sp.* and *Seirpus sp.* used in this coastal Hydraquent paddy soil nearby LERD, grew healthily and help remove $\text{NH}_4\text{-N}$ mineralized from the sewage and $\text{H}_2\text{PO}_4\text{-P}$ released, probably from FePO_4 reduction, after harvesting the plants. Later investigation assured that the 7 days cycle (5 days flooding and 2 days drying) each gave no difference from the former 10 days one and is well accepted in Thailand (Worcester Polytechnic Institute, 2012). Glucose should be the best electron donor and best stimulator for both aerobic and anaerobic decomposition of the OC. Simulation of flooding period of completely aerobic soil in the AFDSP system by anaerobic incubation of the excess glucose solution in a soil for sufficient time till the maximum anaerobic metabolism has been reached should be the treatment potential of the soil for the OC and other high BOD urban sewage. Excess glucose solution load at 3,000 ppm or 3,000 mg/kg soil or 400 me OC/kg soil is calculated from some data of Prabuddham (1975), that is, $\text{M\&J-Fe(III)} = 0.2839 \text{ Fe total}$ and $\text{Fe total} = 0.35 + 0.06 \text{ clay}$. For the most production paddy soil Typictropaquet (Rb series) (which is always used for urbanization) having 52% clay, should have 0.985% Fe(III) or 176.4 me Fe(III)/kg soil comparing with the 400 me OC/kg mentioned. Since not only soil electron acceptors, but also other microbial controlling activities available in soil (Alexander, 1961) should affect the soil potential treatment of OC, by this AFDSP, investigation in various soil subgroups for wider applicability, should be, therefore, worth studying.

2. Materials and Method

2.1 Preliminary Experiment

In this study, 10 grams of the soil, 20 ml of 1,500 mg/l glucose solution without and with 3,750 mg/l of 16-20-0 chemical fertilizer in 180ml flat with screw cap PE bottle to assure complete metabolic activities of soil decomposer, 5x2 factorial in completely randomized design (CRD) design of 5 periods of incubation (P): 0, 1, 3, 5 and 7 days and 2 fertilizer levels: without(0) and with fertilizer(+), with 3 replications, in a Rb soil series (Tropaquets2), has been carried out.

At the sampling time, the excess glucose solution was determined colorimetrically by the phenol-sulfuric acid method (Dubois et al, 1956), after 20 ml H_2O adding, 30 minute horizontally shaking, 10 minute 13,000 rpm centrifuging, by spectrophotometer at 420 nm wavelength. The glucose treated was obtained by subtraction technique and statistically analyzed by the method described by Gomez and Gomez (1984), as shown in Table 1.

Table 1. Effect of incubation period (P) and chemical fertilizer (F) and its interaction (P x S) on glucose treated (ppm)

Chemical Fertilizer (F)	Incubation period days (P)					Pure effect of F
	0	1	3	5	7	
without	0 t	1,893 s	2,330 q	2,730 p	2,730 p	1,935 i
with	0 t	0 t	2,160 r	2,630 p	2,650 p	1,488 j
Pure effect of P	0 d	947 c	2,245 b	2,680 a	2,690 a	grand mean 1,712

F.F.(1,20)= 401.64**; P.F(4,20) = 2,283.82**; P x S.F(4,20) = 263.70**

Error MS = 3,720; CV = 3.56%

Highly significant difference ($P < 0.01$) were observed in all factors studied, that is; (a) pure effect of 5 periods of incubation highly significantly increase the glucose treatments in the order $2,690 a = 2,680 a > 2,245 b > 946 c > 0 d$ ppm for the 7, 5, 3, 1 and 0 days of incubation respectively; (b) highly significantly difference between without (1937 i) and with chemical fertilizer (1,488 j) and (c) highly significantly difference in P x F interaction where at the 1 and 3 days treatments very strong negative effect of fertilization were observed.

Increase anaerobic treatment of the glucose added toward the incubation periods confirmed worldwide reports (Fuchs et al., 2003; Alkarimiah et al., 2011). Inferior of the added chemical fertilizer, observed during the first 3 days, especially after 1 day incubation might be induced by the arisen osmotic pressure minimized osmosis of water into the microbial cell and inhibited temporarily the soil facultative aerobic activities, however, the negative effect was rapidly recovered reaching the treatment potential since 5 days of incubation.

Nevertheless, some soils in the main experiment have been lower nutrient level than this soil. Therefore, 5 days anaerobic incubation with chemical fertilizer added be plausible for the potential treatment of glucose by the soil.

2.2 Main Experiment

2.2.1 Materials and Methods

The Tropaquets used and other 16 soil Greatgroups described and mapped by Land Development Department (LDD) of Thailand, representing possible urbanization now and in the future were selected. Edaphic physical and chemical properties of surface soils, and the methods used are shown in Table 2.

Table 2. Methods used for the surface soil properties

Soil properties	Analysis methods
Soil texture	Jackson (1958)
Electro conductivity : (EC)	Jackson (1958)
pH	Jackson (1958)
Exchangeable potassium (Exc. K^+)	United States Department Agricultural (2009)
Exchangeable calcium (Exc. Ca^{++})	United States Department Agricultural (2009)
Exchangeable magnesium (Exc. Mg^{++})	United States Department Agricultural (2009)
Cation exchange capacity : (CEC)	United States Department Agricultural (2009)
Organic matter (O.M.)	Walkley & Black (1934)
Bray II - P (ppm)	Bray and Kurtz (1945)
Amorphous Mn(IV) (A&K-Mn)	Asami and Kumada (1959)
Crystalline & Amorphous Fe(III) (M&J Fe)	Mehra and Jackson(1960)
Amorphous Fe(III) (Mc&D-Fe)	McKeague and Day (1966)

Similar incubation technique in the first experiment was also used but, all of the 17 soils were incubated only for 5 days, the potential treatment periods proved and using only the fertilized glucose solution to assure sufficient

nitrogen and phosphorus for other soil of lower fertility status owing to complete recovery at the period verified. This CRD main experiment of 17 soils in 3 replications was carried out. The total Fe(II) produced (FeII) from the soil Fe(III) reduction in the anaerobic soil residue in the 17 soils, extracted by 1N HCl (Amari & Mengel, 2006; Bodegom et al., 2003; Christensen et al., 2000). The glucose treated which is the potential treatment of each soil of the 17 ones were the most important subject were determined by Atomic Absorption Spectrophotometer. AAS matter needed by the same method used earlier. Analysis of variant of the potential glucose treatment by method described by Gomez and Gomez (1984) was also performed. Simple linear relationship of the soil factors (X_i) and the glucose treated (Y) by Pearson 1 tailed correlation and linear multiple regression with Stepwise and Enter method of the selected significant soil factors (Hoyle, 1995; Tabachnick & Fidell, 2007).

3. Results and Discussions

3.1 Properties of the Surface Soils

Tables 3 and 4 present the selected properties of the 17 surface soils and summarized statistics of them respectively. The selected soil samples introduced in Table 3, should be universally accepted because 7 soil Orders out of the 9 and 12 Thai and world ones that is, Alfisols (4), Inceptisols (3), Mollisols (3), Ultisols (3), Vertisols (2), Spodosols (1) and Oxisols (1) have been used. Not only the upland soils (11) but also the aquatic moisture regime lowland paddy soils (6) have been selected. Nevertheless, the clay content, the most important ecological impact was arranged orderly. Wide variation (>50% CV) distinguished, in the sand, soil CEC, basic cations and the possible electron acceptors have been expected because of the different soil Orders. Moderate variation (>20-<50% CV) might be due to our small humid tropical country. Moderately narrow variation (<20% CV) in the pH and EC might come from our clayey soil buffering capacity for the pH and saline soils of the Northeast regions and that of the coastal areas have been omitted respectively. Since most of the soils studied had $51 \pm 23\%$ clay, this inorganic, soil fertility should have high retention of ions, owing to their negative charges (CEC) and their capillary force. The humus organic colloid, though having higher CEC, might play less important role on soil fertility status because of its much less abundant (Stevenson, 1982).

Table 3. Physical and chemical properties of the 17 Thai surface soils used

Great groups	Sand	Silt	Clay	EC	pH	Exc.K	Exc.Ca	Exc.Mg	O.M	O.N.	CEC	Bray II-P	M&J-Fe	Mc&D	A&K-Mn
	(%)	(%)	(%)	(mS/cm)		(ppm)	(ppm)	(ppm)	(%)	(ppm)	(me/100g)	(ppm)	(ppm)	(ppm)	(ppm)
Pelluderts	5	16	79	0.2	4	142	4,380	720	3.58	1,790	54.5	40	20,477	15,200	250
Calcistolls	4	20	76	0.28	7.4	42	9,520	420	2.82	1,410	50.6	23	20,670	2,700	945
Haplustolls	3	22	76	0.09	6.9	126	8,120	280	1.94	970	45.1	62	25,926	12,600	975
Haplorthoxs	3	23	74	0.05	4.3	130	1,680	260	2.85	1,425	35.2	131	76,298	38,200	1,165
Haplaquolls	9	19	72	1.6	3.3	134	1,460	1,160	2.48	1,240	37.4	10	13,401	7,200	55
Tropaquepts1	2	27	71	0.2	5.5	282	2,980	1,260	4.39	2,195	34	19	14,802	9,300	110
Plinthaquults	1	31	68	0.03	5.2	8	280	80	2.04	1,020	20.8	5	9,872	7,500	75
Tropaquepts2	14	20	66	0.47	5.2	274	5,600	560	1.74	870	29.1	76	18,637	5,200	155
Pellusterts	1	40	59	0.24	4.3	86	2,360	360	3.12	1,560	33	62	23,966	17,015	165
Tropaqualfs	25	35	40	0.1	5.2	8	1,220	540	2.33	1,165	21.4	24	13,453	9,000	515
Haplustalfs	16	46	38	0.05	5.5	54	2,040	240	1.91	955	17.7	70	13,165	5,700	355
Paleustalfs	40	28	32	0.06	5.2	102	1,480	140	1.89	945	14.1	34	2,717	5,700	420
Natraqualfs	42	29	29	0.04	5.5	14	820	180	1.15	575	10.3	5	6,651	3,700	130
Paleustults	34	39	27	0.05	5.5	102	700	160	1.65	825	14	24	11,140	2,600	255
Plinthustults	57	16	27	0.01	5.6	40	240	60	1.17	585	5	6	13,758	1,500	75
Tropohumods	80	6	14	0.01	4.8	32	60	140	1.36	680	10.1	4	3,832	400	5
Dystropepts	70	18	13	0.02	4.9	31	380	100	0.81	405	5.3	3	2,862	80	15

As shown in Table 5, positive linear relationship influenced by the soil clay (%) and CEC at significant levels and higher were observed in all macronutrient parameters and the electron acceptors. The positive linear relationship affected by the soil O.M. at the highly significant for the Exc. Mg^{++} and significant for the Mc&D-Fe were also observed. The rest had also positive trends of their relatively high r values were also distinguished. These positively linear relationship indicated that these colloids positively controlled the soil fertility not only for the producers but also the soil decomposers.

Table 4. Summarize surface soil properties of the 17 Thai soils

Soil properties	Parameters					
	Max.	Min.	M.P	X	SD	CV (%)
a. General physico-chemical properties						
Sand (%)	80	1	40	24	25	105
Silt (%)	46	6	26	26	10	39
Clay (%)	79	13	46	51	23	46
CEC (me/100g)	54.51	4.95	28.73	25.72	15.13	59
pH	7.4	3.3	5.4	5.2	0.9	18
EC (mS/cm)	1.6	0.01	0.8	0.21	0.37	18
O.M. (%)	4.39	0.81	2.6	2.13	0.85	40
b. Organic nitrogen (O.N.) and some nutrients						
O.N. (ppm)	2,195	405	1,360	1,095	454	41
Bray II-P	131	2	65	35	35	100
Exc. K ⁺ (ppm)	282	8	145	95	81	85
Exc. Ca ⁺⁺ (ppm)	9,520	60	4,790	2,548	2,717	107
Exc. Mg ⁺⁺ (ppm)	1,260	60	660	392	349	89
c. Possible electron acceptors						
M&J-Fe (ppm)	76,298	2,717	39,508	17,155	16,265	95
Mc&day (ppm)	32,000	80	19,140	8,928	9,477	106
A&K-Mn (ppm)	1,165	5	585	333	352	106

Table 5. Relationship between the soil colloids and CEC on exchangeable bases (except Na⁺), Bray II-P and the possible electron acceptors in the 17 soils

Dependent Variable (Y _j)	Independent variable (X _i)		
	Clay (%)	O.M. (%)	Soil CEC
a. Macronutrients			
O.N. (ppm)	0.737**	1	0.747**
BrayII-P (ppm)	0.441*	0.287 ^{ns}	0.396 ^{ns}
Exc. K (ppm)	0.515*	0.537*	0.442*
Exc. Ca (ppm)	0.647**	0.355 ^{ns}	0.771**
Exc. Mg (ppm)	0.570*	0.727**	0.568**
b. Possible electron acceptors			
M&J-Fe (ppm)	0.539*	0.388 ^{ns}	0.468*
Mc&D (ppm)	0.559**	0.517*	0.483*
A&K-Mn (ppm)	0.451*	0.234 ^{ns}	0.515*

Table 6. Glucose treatment potential and the Fe(II) produced in the 17 Thai soils

Great groups	Glucose treated ppm or gram/ton	Fe(II) produced ppm or gram/ton
Haplustolls	2,573 a	1,050 h
Calciustolls	2,533 a	6,693 bc
Haplaquolls	2,447 b	4,753 e
Tropaquepts1	2,400 bc	3,540 f
Haplustalfs	2,367 c	5,123 e
Tropaquepts2	2,287 d	7,017 a
Pellusterts	2,187 e	6,340 c
Tropaqualfs	2,180 e	7,290 a
Haplorthoxs	2,107 f	1,057 h
Pelluderts	2,040 f	5,610 d
Dystropepts	1,933 g	201 k
Paleustalfs	1,893 g	607 ijk
Natraqualfs	1,707 h	847 hij
Plinthaquults	1,520 i	1,997 g
Plinthustults	1,520 i	953 hi
Paleustults	933 j	530 jk
Tropohumods	933 j	373 k

Glucose treated $F = 589.9^{**}$; MS error 1,913.13; CV = 2.3%.

Fe(II) produced $F = 416.29^{**}$; MS error 52,911.7; CV = 7.2%.

Pearson correlation (1-tailed) study of this anaerobic decomposition of glucose treated or dependent variable y .

3.2 Glucose Treatment Potential of the 17 Soils

Highly significant difference ($P < 0.01$) in the glucose treated at 5 days anaerobic incubation which should be the potential treatment of the organic sewage and in the Fe (II) compounds produced from the reduction of the Fe(III) in the soil prior to flooding, which were arranged in the order of the treatment potential presented in Table 6, were clearly observed. The order of The orders of the glucose treated were; Haplustoll (2,573 a) = Calciustolls (2,533 a) > Haplaquolls (2447 b) \approx Tropaquept1 (2,400 bc) \approx Haplustalfs (2,367 c) > Tropaquept2 (2,287 d) > Pellusterts (2,187 e) = Tropaqualfs (2,180 e) > Haplorthoxs (2,107 f) = Pelluderts (2,040 f) > Dystropepts (1,933 g) = Paleustalfs (1,893 g) > Natraqualfs (1,707 h) > Plinthaquults (1,520 i) = Plinthustults (1,520 i) > Paleustults (933 j) = Tropohumods (933 j). The arrangement order of the Fe(II) produced were; Tropaqualfs (7,290 a) \approx Tropaquepts2 (7,012 ab) \approx Calciustolls (6,693 bc) > Pelluderts (5,610 d) > Haplustalfs (5,123 e) = Haplaquolls (4,753 e) > Tropaquepts1 (3,540 f) > Plinthaquults (1,997 g) > Haplorthoxs (1,057) = Haplustolls (1,050 h) \approx Plinthustults (953 hi) \approx Natraqualfs (847 hij) \approx Paleustalfs (607 ijk) \approx Paleustults (530 jk) \approx Tropohumods (373 k) \approx Dystropepts (201 k) respectively.

It can also be simplified from this table that the glucose treated range was 933-2,5733 ppm but most of them distributed within the range of $1,974 \pm 596$ ppm with the CV of 25.6% which was less variation than that of almost all of that of the soil properties, (Table 4), excepted that of the pH and EC. The Fe(II) produced range was 201-7,290 ppm which most of them distributed within the range of $3,175 \pm 2,710$ ppm with much higher CV(%) at 85.3%. Table 6 clearly presented that Thai soils and probably elsewhere in humid tropical countries, can treat urban wastewater using Fe(III) compounds as electron accepters during flooding, in the AFDSP system up to the maximum of 2,573 ppm glucose. Since glucose has 40% OC which has 3 equivalent weight, the maximum glucose treated should be 343.1 me/kg, which will equivalent to = 343.1 meO₂/kg from the BOD_{5,20} or 2,745 mgO₂/kg. Since be the approximate recovery of the sewage load is about 90% (Tantanasarit, 1995). Possible BOD load of the sewage that can be treated by this AFDSP should be not less than 3,049 mgO₂/kg of the soil.

The actual BOD load should be higher than this because, aerobic decomposition of the sewage during 2 days drying periods in each cycle of the AFDSP system, is ignored.

Pearson correlation (1-tailed) study of this anaerobic decomposition of glucose treated or dependent variable y and the Fe(II) produced, was highly significantly correlated ($R^2=0.358$, $n=17$) and the y could be predicted by the Equation 1

$$y(\text{ppm}) = 1,619 + 0.1 \text{ Fe(II) produced (ppm)} \quad (1)$$

High intercept of Equation 1, 1,619 ppm or 9 mmol of glucose treated in each kilogram of soil without Fe(II) production, clearly indicated that the glucose had been treated by Thai soils prior to Fe(III) reduction. Activities on fermentation process in this glucose-soil system by indigenous yeast might play important role prior to the Fe(III) compounds reduction.

When forecasted glucose treated (y) with selected soil properties giving highly significant and significant influence from the Pearson correlation 1-tailed study such as; %Clay, %Sand, %O.M., CEC, Exc.Ca, Exc.Mg and A&K-Mn (Table 7) for the multiple relationship by Stepwise linear regression method, %Clay only gave highly significant correlation ($R^2=0.433$, $n=17$) and the glucose treated (Y) could be predicted by Equation 2

$$y(\text{ppm}) = 1,266 + 14 \% \text{Clay} \quad (2)$$

Table 7. Correlation coefficients (r) of the glucose treated and soil properties

Independent variable	Pearson correlation parameter		
	r	a	b
a. General physico-chemical properties			
Sand (%)	-0.655**	2,281	-12.9
Silt (%)	0.116 ^{ns}		
Clay (%)	0.658**	1,266	14
CEC (me/100g)	0.652**	1,430	21.2
pH	0.134 ^{ns}		
EC (mS/cm)	0.394 ^{ns}		
O.M. (%)	0.494*	1,389	267
b. Organic nitrogen (O.N.) and some nutrients			
O.N. (ppm)	0.494*	1,389	0.5
Bray II-P	0.403 ^{ns}		
Exc. K ⁺ (ppm)	0.389 ^{ns}		
Exc. Ca ⁺⁺ (ppm)	0.643**	1,678	0.1
Exc. Mg ⁺⁺ (ppm)	0.544*	1,674	0.8
c. Possible electron acceptors			
M&J-Fe (ppm)	0.307 ^{ns}		
Mc&day (ppm)	0.315 ^{ns}		
A&K-Mn (ppm)	0.435*	1,772	0.6
d. Fe(II) produced(ppm)	0.598**	1,619	0.1

The increase of R-square from 0.433 by the multiple linear regression to 0.653 by Enter method clearly indicated superiority of this method. x_1 , x_2 , x_3 , x_4 , x_5 and x_6 are respectively. Clay (%), CEC (me/100g soil), Exc.Ca⁺⁺ (ppm), Exc.Mg⁺⁺ (ppm), OM (%) and A&K-Mn (ppm) respectively. Then the forecasted by Equation 3 ($R^2=0.653$, $n=17$)

$$\text{Glucose treat}(y, \text{ppm}) = 1,381.202 + \{8.816(x_1) - 17.010(x_2) + 0.086(x_3) + 0.825(x_4) - 49.321(x_5) + 0.449(x_6)\} \quad (3)$$

Table 8. Predicted glucose potential treatment (ppm) and their error (%)

Great groups	By Equation 1			By Equation 2			By Equation 3		
	Predicted	Error	Grade	Predicted	Error	Grade	Predicted	Error	Grade
Haplustolls	1,735	-32.6	F	2,330	-9.4	B ⁺	2,555	-0.7	A
Calcistolls	2,355	-7.0	B ⁺	2,330	-8.0	B ⁺	2,641	4.3	A
Haplaquolls	2,142	-12.5	B	2,274	-7.1	B ⁺	2,365	-3.4	A
Tropaquepts1	2,008	-16.3	B	2,260	-5.8	B ⁺	2,557	6.5	B ⁺
Haplustalfs	2,183	-7.8	B ⁺	1,798	-24.0	F	1,854	-21.7	F
Tropaquepts2	2,391	4.5	A	2,190	-4.2	A	2,395	4.7	A
Pellusterts	2,316	5.9	B ⁺	2,092	-4.3	A	1,751	-19.9	B
Tropaqualfs	2,421	11.1	B	1,826	-16.2	B	2,037	-6.6	B ⁺
Haplorthoxs	1,735	-17.6	B	2,302	9.3	B ⁺	2,176	3.3	A
Pelluderts	2,236	9.6	B ⁺	2,372	16.3	B	2,057	0.8	A
Dystropepts	1,641	-15.1	B	1,448	-25.1	F	1,488	-23.0	F
Paleustalfs	1,686	-10.9	B	1,714	-9.5	B ⁺	1,762	-6.9	B ⁺
Natraqualfs	1,712	0.3	B ⁺	1,672	-2.1	A	1,682	-1.5	A
Plinthaquults	1,839	21.0	F	2,218	45.9	F	1,650	8.6	B ⁺
Plinthustults	1,724	13.4	B	1,644	8.2	B ⁺	1,580	3.9	A
Paleustults	1,677	79.8	F	1,644	76.2	F	1,606	72.1	F
Tropohumods	1,660	77.9	F	1,462	56.7	F	1,389	48.9	F

Validity of this 3 equations developed was also evaluated by the proposed acceptable criteria: A, B⁺, B and F grades for the 0-5, 5.1-10, 10.1-20 and over 20.1 error percentages respectively illustrated in Table 8. It can be seen that all of them could be acceptable of; A grades: Equation 3(8) > Equation 2(3) > Equation 1(2); in the orders of A+B⁺ grade: Equation 3(12) > Equation 2(10) > Equation 1(6). Applicable suitability of equations developed should not be evaluated simply by its highest R² value, but other 3 parameters; economy, simplicity and rapidity of the whole process should also be considered. Equation 3 might be recommended by developed humid tropical countries, but Equation 2 should be recommended for all developing ones because of its better economic, simplicity and rapidity advantages over that of the Equation 3. Though Equation 1 confirmed importance of the active Fe(III) compounds, but it is unnecessary since direct determination of the OC treatment potential of the soil could be obtained by this anaerobic incubation technique.

4. Conclusion

These summarized results were; (a) The glucose treated range was 933-2573 ppm; (b) The glucose treated highly correlated with the Fe(II) produced; (c) Simple relationship studies between the glucose treat (y) highly significant correlation with such soil properties as clay(x₁), CEC (x₂) Exc.Ca(x₃) and significantly correlated with such ones as Exc.Mg(x₄), OM(x₅) and A&K-Mn(x₆); (d) Multiple relationship studied by Stepwise regression analysis showed that only the clay content played the important role, but by the Enter method improved the R square 0.433 to 0.653.

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