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Evaluation of Phytotoxicity effect of palm oil mill effluent and cassava mill effluent on tomato (*lycopersicum esculentum*) after pretreatment options

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***Abstract*—Agro industrial effluents' management such as Palm Oil Mill Effluent (POME) and Cassava Mill Effluent (CME) have been a major environmental concern in countries producing them. These effluents are land and aquatic pollutants when discharged untreated, due to presence of high organic load and their phytotoxic properties. Pretreatment measures comprised of phase separation involving sedimentation, aeration to enhance biodegradation and pH neutralization. A randomized complete block design experiment in factorial arrangement was set up to assess effects of aeration, settling and pH neutralization on POME and CME phytotoxicity on tomato (*Lycopersicum esculentum*) germination and seedling development. Results obtained showed that aeration was the most significantly effective pretreatment technique for POME and CME. Phytotoxicity decreased when effluents were left to aerobically decompose for 6 days. pH neutralization increased phytotoxcity in the two effluent streams. Settling did not significantly reduce phytotoxicity in CME but did in POME. The 3-way Interaction was not significant in all the parameters measured. Management plans for these effluent streams should consist of well designed pond system, metal tanks equipped with blowers for proper decomposition before disposal.**

I. Introduction

Agro industrial effluents' management have been a major issue of environmental concern globally. Agro industrial effluents include Cassava Mill Effluent (CME), Palm Oil mill Effluent (POME), Olive Mill Effluent (OME) etc. These effluent streams are serious nuisance when discharged untreated, as they have high levels of organic loads and some organic acids [1]. The phytotoxic properties of these effluents can be attributed to high concentration of polyphenols which are known to possess antibacterial properties [2]. Besides its phytotoxic properties, they are amenable to biodegradation. Great amount of POME and CME are produced in tropical climate regions with attendant management problem.

A number of effluent treatment methods have been employed in recent years and these are broadly grouped into physical, chemical and biological. Chemical method is based on effluent treatment with flocculants and coagulant, cryogenesis, ultra-filtration, reverse osmosis, thermal concentration and evaporation in ponds. The cost implications of these methods are very prohibitive and may not completely solve the problem, because of the need to dispose the sludge derived from the process [3]. Consequently, biological methods are based on production of proteins, poly-hydroxy-β-butyrates, poly-hydroxy-alcanoates and exopolysaccharides, anaerobic digestion and composting [4], [5], [6], [7]. This method is far beneficial since it is less expensive and the by-product is utilized in agricultural production .

These effluent streams are normally disposed of in drainage channels or stored in evaporation ponds or worse still discharged in arable lands to possibly avert the cost of treatment. This practice is predominant in developing countries where effluent discharge standards are not strictly adhered to.

Common pre-treatment techniques prior to plant application or disposal could consist of phase separation through a settling basin, aeration to promote biological degradation and pH neutralization. Dilution with water, although not often considered as a waste water treatment technique, can nevertheless, be an inexpensive low budget technology to be adopted by the small sized mills. Palm oil mill effluent and cassava mill effluent phytotoxicity is a complex property, since more than one compound is responsible for it. Polyphenols are not necessarily the sole compounds responsible for phytotoxicity [8], [9]. There could be volatile organic acids, alcohols, aldehydes and other smaller molecules responsible for phytotoxicity in POME and CME. Presence of cyanogenic glucosides (mostly linamarin) in CME responsible for toxic effects in humans and livestock are well documented [10], [11]. [1] reported that phytotoxic properties can also be related to low pH and salts, in addition to phenols. There was also an indication of alteration of soil properties (e.g. competitive sorption effect of certain ions, alteration of cation exchange capacity) following soil application of POME [12], [13].

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The growth inhibitions of different vegetables after POME and CME application to soil have been observed [14]. This inhibition has been studied using seed germination and early development of different vegetables in a screen house experiment under different concentrations of the effluent [14]. Results indicated an inhibitory effect on seed germination and also in early plant development when varied concentrations of these effluents were applied.

This present study was undertaken to investigate the effect of three pre-treatment techniques viz; aeration, settling and pH neutralization, prior to POME and CME disposal, on seed germination and early plant growth using tomato seeds (*lycopersicum esculentum*).

II. Materials and Methods

A 15 L POME composite sample was collected from ADAPALM oil mill, a government owned centrifugal palm oil mill in South eastern, Nigeria in 2008 and was refrigerated at 3^{o}C. Similarly, 10 L composite sample of Cassava mill effluent was obtained locally from cassava processing mills using hydraulic press pumps in South eastern, Nigeria and was also stored at 3^{o}C, prior to analysis. POME was filtered through 5 mm sieve to remove heavy suspended particles.

The POME and CME were analysed for total suspended solids (TSS), chemical oxygen demand (COD), biochemical oxygen demand (BOD_5), pH and electrical conductivity. All the parameters analysed were in duplicates. Chemical oxygen demand determination was based on closed reflux dichromate oxidation colorimetric method and was read out in DR 2000 HACH® spectrophotometer as explained in detail in [15]. Samples for BOD_5 measurements were prepared according to a modified method explained in [15]. 2-Chloro-6 trichloro-methyl pyridine was used to inhibit nitrification as stipulated by the procedure. The apparatus used was the Lovibond BOD IR-sensomat, which consists of an IR-pressure sensor acting as the measurement device, BOD- sensomat and stirring system. Each sample was collected in a 500 ml BOD flask and was filled completely and covered satisfactorily with foil cap and left for a 5-day period. The resultant carbondioxide from microbial respiration is absorbed with potassium hydroxide (KOH), which creates a decrease of the air pressure in the BOD flask. The pressure decrease is detected by the IR-sensor, logged into the BOD-sensor and converted directly in mg/L of BOD. Total suspended solid (TSS) was determined gravimetrically by evaporating to dryness 100 ml of unfiltered sample (effluent) and heating to constant weight. The weight difference was expressed in mg/l. The pH was measured using a portable pH meter (model (pH 95) by WTW®) in an aqueous solution (1:5 in effluent) and electrical conductivity (EC) was measured using Sension5 conductivity meter by HACH®. All conductivity measurements were referred to a 25^{o}C temperature.

A. Pre-treatment description

Pre-treatment techniques implored were as follows;

*1) Settling:*Settling is a common physical separation technique that can be practised prior to further treatment of POME and CME to reduce TSS concentration. Preliminary settling conducted in this study indicated that TSS concentration reached a stable value after a 5-day retention time.

*2) Aeration:*POME and CME aeration can enhance the aerobic decomposition of certain potentially phytotoxic compounds, such as polyphenols and organic acids. Aerobic decomposition can break down several organic compounds to intermediate organic metabolic by products essential for plant growth or oxidize some components to volatile constituents e.g. CO_2.

3) pH adjustment: The initial pH of POME and CME can range from 4.5-5.5 [13] and 4.2-6.7, respectively. Neutralization of POME and CME has been suggested as a pretreatment technique prior to other downstream techniques such as biological treatment. In practice, neutralization of acidic POME can be achieved through the use of lime or soda ash, followed by the formation of precipitants. The advantage of using alkaline chemicals is that the settling retention time is much shorter than the time required when sedimentation is induced to occur by the forces of gravity.

B. Experimentation

POME and CME phytotoxicity was studied on tomato seeds (*lycopersicum esculentum*) obtained from national seeds Umudike Umuahia. Phytotoxicity was measured using a modified Zucconi test [16] by measuring seed germination and development after 3 weeks. Twenty seeds were placed on filter papers (Rund filter, MN 615, 11cm) installed in glass Petri dishes with dimensions 110 mm x 20 mm. 10 ml of POME and CME samples were respectively and uniformly added to each dish. The dish was kept in a dark incubator at 26±2^{o}C for 5 days. The control consisted of 10 ml of distilled water. All the treatments were in triplicates. All seeds were previously soaked for 12 h in distilled water before commencement of the experiment to accelerate seed growth.

A germination index (GI) was calculated by accounting for the number of grown seeds and the average sum of seeds' root elongation in a sample as related to the control [16]. Results were expressed as a percentage of the control.

$$GI = \frac{\text{number of grown seeds in sample}}{\text{number of grown seeds in control}} \times \frac{\text{average sum of root lengths in sample} \times 100}{\text{average sum of root length in control}} \tag{1}$$

A seed was considered grown when its root lengths exceeded 5 mm. For the root lengths less than 5mm, the root length was equal to 0 and the seed was not considered grown. The average sum of root length comprised the sum

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of the lengths of all grown seeds in a Petri dish.

The experimental design was set up as a full factorial design with three factors at two levels {low (-1) and high levels (+1)} for the two effluent streams. The levels at which the three factors were set are presented in Table 1.0. The full factorial experimental design was seven combinations excluding control. All treatment units had three replicates.

Settling was achieved by allowing 100 ml each of the effluents (POME and CME) to stand for maximum of 5 days. Aeration was achieved through stirring by adding approximately 300 ml of POME and CME samples to a 1 litre flask each. This was stirred daily for a 6-day period. The beaker remained open to the atmosphere during stirring to facilitate air diffusion into the samples. Evaporated water was replenished using distilled water. pH was adjusted to 7 by using a 1 M $NaHCO_3$ solution (when the initial pH was lower than 7) or a 0.1 N H_2SO_4 solution (when the initial pH was higher than 7). pH was always adjusted after settling and aeration pretreatments. Control consisted of seed with distilled water. The result of the control was used as the basis to calculate the GI index (Table 1.0). Other data collected were dry weight (DW) and height of tomato seedling after 3 weeks duration of the experiment.

TABLE 1.0 SUMMARY OF THE FACTORS AND LEVELS USED IN THE STUDY.

Factors	Low level (-)	High level (+)
Settling (ST)	Unsettled	Settled for 5 d
Aeration (AE)	Non aerated	Aerated for 6 d
pH	No pH adjustment	pH adjusted to 7

C. *Statistical analysis*

Analysis of variance was used on the full factorial design with (α=0.05). This was done with GenSat discovery edition 3. Measured parameters were calculated using the equation below

$$\partial\rho = n + (X_{st} / 2) L_{st} + (X_{ae}/2) L_{ae} + (X_{pH}/2) L_{pH} + (X_{stae}/2) L_{st}L_{ae} + (X_{stpH}/2) L_{st} L_{pH} + (X_{aepH}/2) L_{ae} L_{pH} + (X_{st\,ae\,pH}) L_{st} L_{ae} L_{pH} + \varepsilon \quad (2)$$

$\partial\rho$ = experimental response (e.g. GI), n= experimental mean, X_{st}= effect of settling, $X_{ae=}$ effect of aeration , $X_{pH,=}$effect of pH, $X_{st\ ae}$= effect of settling and aeration interaction, $X_{stpH=}$effect of settling and pH interaction, X_{aepH}= effect of aeration and pH interaction, $X_{st\ ae\ pH=}$ effect of settling, aeration and pH interaction. L_{st}, L_{ae} and L_{pH} are the low or high applications corresponding to values of - 1 and + 1, respectively, of each of the three factors used in the experiment, ε are the errors or residuals that are normally distributed with zero mean and constant variance [17].

III. RESULTS AND DISCUSSION.

POME and CME phytotoxicity have been attributed to the phenolic and organic acid (e.g. acetic and formic acids) content. These compounds are often produced along with other microbial metabolites during POME and CME storage. Reduction of the above constituents by any means will eventually reduce phytotoxicity. Initial chemical determinations on POME and CME indicated substantial levels of suspended solids, organic residuals and are acidic in nature (Table 2).

TABLE 2.0 CHEMICAL CHARACTERISTICS OF CME AND POME

Effluent[a]	BOD_5 (mg/l)	COD (mg/l)	TSS (mg/l)	EC (dS m^{-1})	pH
POME	16524.2±34	13634.0±23	11734±45	0.19 ± 0.021	4.5 ± 2.1
CME	11230±56	976±12	542±23	0.12±0.02	5.6±2.4

POME= palm oil mill effluent, CME= cassava mill effluent, BOD= biochemical oxygen demand, COD= chemical oxygen demand, TSS= total suspended solid. EC= electrical conductivity, [a] mean ± SD.

Aeration was observed to be most important technique affecting phytotoxicity. Aeration significantly affected all measured parameters in both POME and CME (Tables 3 and 4). Aeration apparently reduced BOD concentration through biological decomposition induced by resident microbial community present in POME as well as in CME. This process transforms several of the phytotoxic compounds to less toxic metabolites and by products such as CO_2. pH neutralization was found to be the least factor affecting POME phytotoxicity. pH neutralization, achieved by adding $NaHCO_3$ salt to both POME and CME rather increased phytotoxicity (Tables 3 and 4 , Eq.3). This is probably attributed to the fact that salts addition increased the POME and CME total dissolved salts (TDS) content, as this was indirectly measured through electrical conductivity measurements. Increased TDS negatively affected germination despite neutral pH, since the sodium ion used in $NaHCO_3$ to raise pH can have a toxic effect on seeds above certain concentration. Therefore, the TDS increase during

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pH adjustment led to increased effluent phytotoxicity. Settling showed significant effect in reducing phytotoxicity in POME but did not effect significant toxicity reduction in CME (Table 4). This is probably because of high solubility of polyphenolic compounds in POME; they go into solution and reduce in concentration. Cyanogenic glucoside compound responsible for phytotoxicity in CME [9], [10] may not have settled as expected within the period left to stand. Aeration and pH interaction significantly ($p<0.05$) affected tomato dry weight in POME as well as in settling and aeration in the number of seeds germinated. However, there was no significant interaction in the tomato grown in CME (Table 4).

A. Discussions based on the model

The magnitude of phytotoxicity can be indirectly determined, since phytotoxicity decreases as other parameters increases. A better understanding of the main effects and potential interactions among the three factors can be obtained after fitting the best reduced mathematical models using GenStat discovery edition 3. The best reduced models are based on a 5 % level of significance (p<0.05).

Tomato seedling best reduced model grown in POME medium

$$GI= 49.8(\pm3.2) + 7.8 (\pm 4.2) L_{st} + 30.9(\pm3.2) L_{ae} -14.5(\pm2.5) L_{pH} +3.5(\pm2.1) L_{stae} - 10.5 (\pm3.2) L_{st\,pH} + 2.4(\pm1.3) L_{ae\,pH} \quad (3)$$

$$Height = 52.6 (\pm4.1) + 23 (\pm2.3) L_{st} + 35.1(\pm2.9) L_{ae} -12.5(\pm 3.4) L_{pH} + 2.4(\pm1.2) L_{stae} - 6.2 (\pm4.5) L_{st\,pH} + 1.2 (\pm0.87) L_{ae\,pH} \quad (4)$$

$$DW = 55.5 (\pm4.5) + 28 (\pm3.2) L_{st} + 32.6 (\pm6.3) L_{ae} - 6.4 (\pm 2.3) L_{pH} + 2.8(\pm1.2)L_{stae} - 2.6 (\pm0.12) L_{stpH} + 2.4 (\pm1.2) L_{aepH} \quad (5)$$

Tomato seedling best reduced model grown in CME medium.

$$GI = 56.4 (\pm3.6) + 40.4(\pm4.2) L_{ae} - 5.6(\pm3.5) L_{pH} + 2.7(\pm 1.4) L_{aepH} \quad (6)$$

$$Height = 60.2 (\pm5.3) + 34.3(\pm3.2) L_{ae} - 4.3 (\pm0.67) L_{pH} + 3.8 (\pm1.6) L_{aepH} \quad (7)$$

$$DW = 58.7 (\pm3.5) + 39.8(\pm4.7) L_{ae} - 3.9 (\pm1.3) L_{pH} + 4.5 (\pm2.3) L_{aepH} \quad (8)$$

Equations (3-5), calculates germination index, plant height and dry weight (DW) as a function of the levels of all pretreatment techniques. From the model, aeration and settling reduced phytotoxcity effect to enhance tomato seed germination, height and biomass production. This finding is consistent with previous works on the use of aerobically digested sludge for irrigation [18],[19], [8], soil amendment [20] and fermentation media [21]. However, it is evident from the model that pH negatively affected all the measured parameters (Eqs.3-5). Similarly, 2 of the 2-way interactions are found to be statistically significant at $p<0.05$ while settling and pH interaction is negative. Three-way interactions, however, is very small and not included in the model

TABLE 3.0 SUMMARY OF ANALYSIS OF VARIANCE FOR % GI, AVERAGE NUMBER OF SEED GERMINATION, AVERAGE TOTAL ROOT LENGTH, HEIGHT, DRY WEIGHT ON PALM OIL MILL EFFLUENT (POME).

Source of variation	Df	% GI		[a]Root length		Number of seed germ.		[b]Height		[c]Dry weight	
		F-stat	*p-value*	*F-stat*	*p-value*	*F-stat*	*p-value*	*F-stat*	*p-value*	*F-stat*	*p-value*
Block	2	2.20	0.45	0.47	0.70	0.37	0.12	1.34	0.13	2.12	0.12
Settling(ST)	1	3.45	0.05	2.41	0.03	1.27	0.02	3.53	0.02	3.43	0.04
Aeration(AE)	1	4.48	0.02	3.71	0.01	0.69	0.01	4.12	0.03	3.45	0.01
pH	1	5.26	0.04	5.21	0.03	4.36	0.04	5.23	0.02	5.67	0.03
ST *AE	1	2.67	0.05	0.91	0.12	1.21	0.03	2.31	0.02	1.95	0.03
ST * pH	1	4.76	0.04	0.61	0.31	2.36	0.13	4.76	0.02	3.41	0.04
AE * pH	1	5.67	0.05	1.71	0.41	1.36	0.36	4.89	0.05	2.32	0.05
ST *AE*pH	1	4.47	0.45	2.18	0.51	2.16	0.16	2.41	0.32	1.34	0.15
Error	14										

[a] average total root length in the petri dish, [b] average total height of the seedling after 2 weeks, [c]average total dry weight of seedling after 2 weeks , df= degree of freedom, GI= germination index.

Aeration is the most significant technique affecting phytotoxicity, since it has the largest coefficients in the model (30.9, 35.1 and 32.6) for GI, height and DW, respectively (see Eqs.3-5). The high coefficients obtained from aeration and settling indicated reduction in phytotoxicity. On the other hand, pH has to be kept low since it can result into increased phytotoxicity. The model is considered adequate, since residuals are normally distributed with zero mean, and constant variance (Fig. 1) and do not have any particular trends when plotted versus predicted values (Fig. 2). Residuals are defined as the difference between model predictions and raw data.

Similarly, equations (6-8) calculates germination, tomato height and biomass production in CME media. Aeration also is the most significant technique affecting phytotoxicity in CME, since it has the largest coefficients: 40.4, 34.3 and 39.8 for GI, height and DW, respectively. pH neutralization rather significantly increased phytotoxicity as was the case in POME media. However, settling technique did not significantly affect phytotoxicity in CME media, since it has very small coefficient and this was excluded from the model. Only aeration and pH 2-way interaction appeared to be statistically significant. The model shown in equation 6-8 is adequate, since residuals are distributed normally with a zero mean. Residuals do not show any particular trend when plotted versus normal score.

Based on the results of the statistical analysis, land disposal of aerobically digested POME and CME can be a potential treatment strategy that can be adopted in some situations in the tropics where production of these effluent streams are considerably high. However, types of soil and groundwater characteristics of the land as well as local water availability should be adequately studied to avoid unhealthy soil reactions. Aeration of the effluents can be achieved via a properly designed tank, with installed aeration equipment, such as blowers. No pH neutralization needs to be practiced and no particular investment in the construction of settling basin needs to be made, since both factors minimally affect phytotoxicity

TABLE 4.0 SUMMARY OF ANALYSIS OF VARIANCE FOR % GI, AVERAGE NUMBER OF SEED GERMINATION, AVERAGE TOTAL ROOT LENGTH, HEIGHT, DRY WEIGHT ON CASSAVA MILL EFFLUENT (CME).

Source of variation	Df	% GI		[a]Root length		Number of seed germ.		[b]Height		[c]Dry weight	
		F-stat	*p-value*	*F-stat*	*p-value*	*F-stat*	*p-value*	*F-stat*	*p-value*	*F-stat*	*p-value*
Block	2	1.21	0.15	0.17	0.10	1.27	0.15	1.65	0.23	1.12	0.14
Settling(ST)	1	2.53	0.12	2.61	0.13	1.37	0.09	3.83	0.22	3.73	0.13
Aeration(AE)	1	5.43	0.02	4.71	0.01	3.69	0.01	4.52	0.01	4.45	0.01
pH	1	5.36	0.03	4.21	0.03	4.66	0.03	5.83	0.02	4.67	0.02
ST *AE	1	3.67	0.33	0.97	0.22	2.21	0.13	2.41	0.15	2.95	0.23
ST * pH	1	3.76	0.37	2.61	0.37	3.36	0.23	5.76	0.34	4.41	0.13
AE * pH	1	4.67	0.03	3.71	0.31	2.36	0.16	3.89	0.02	2.35	0.05
ST *AE*pH	1	3.47	0.55	2.38	0.41	3.16	0.26	3.41	0.22	2.14	0.25
Error	14										

[a] average total root length in the petri dish, [b] average total height of the seedling after 2 weeks, [c]average total dry weight of seedling after 2 weeks , df= degree of freedom, GI= germination index.

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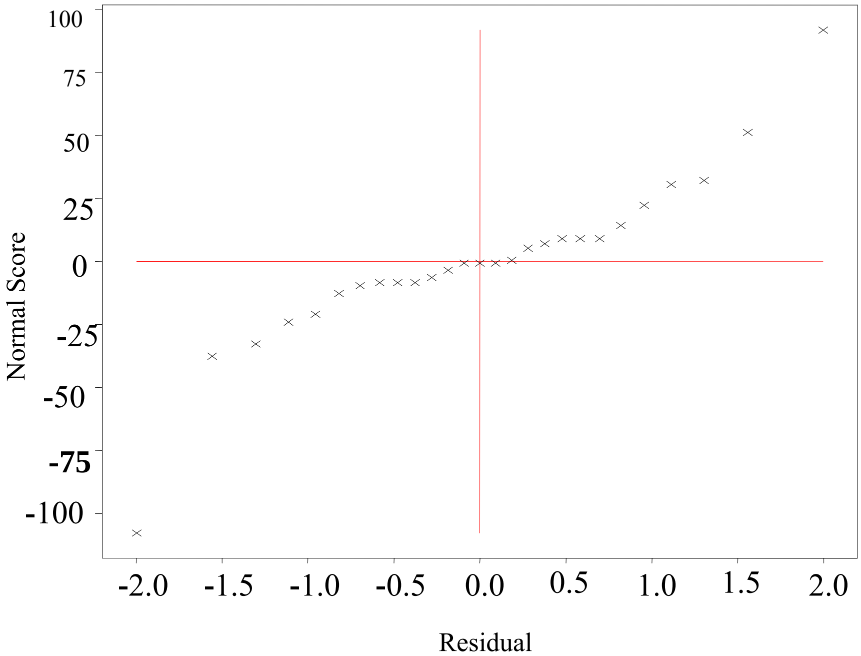

Fig. 1 Normal probability plot of residuals of model (Eqs 3-5)

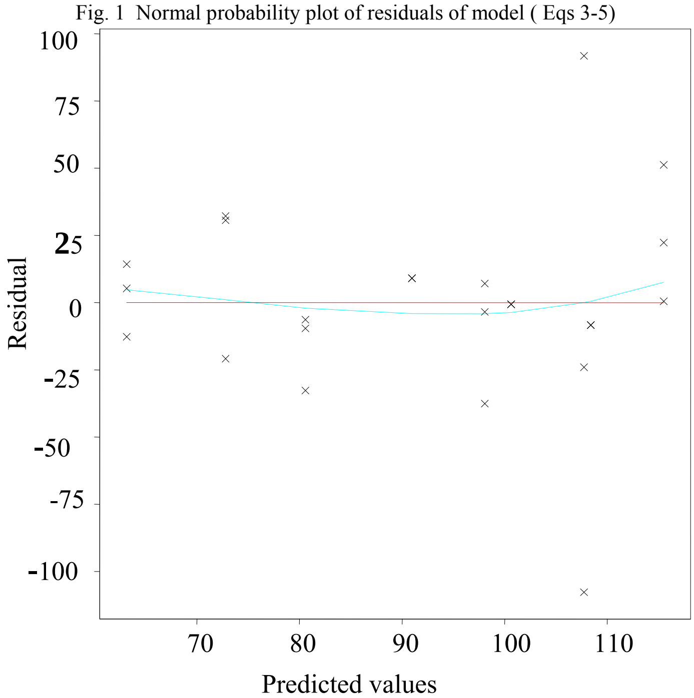

Fig. 2 Residuals versus predicted values for the POME model (Eqs 3-5)

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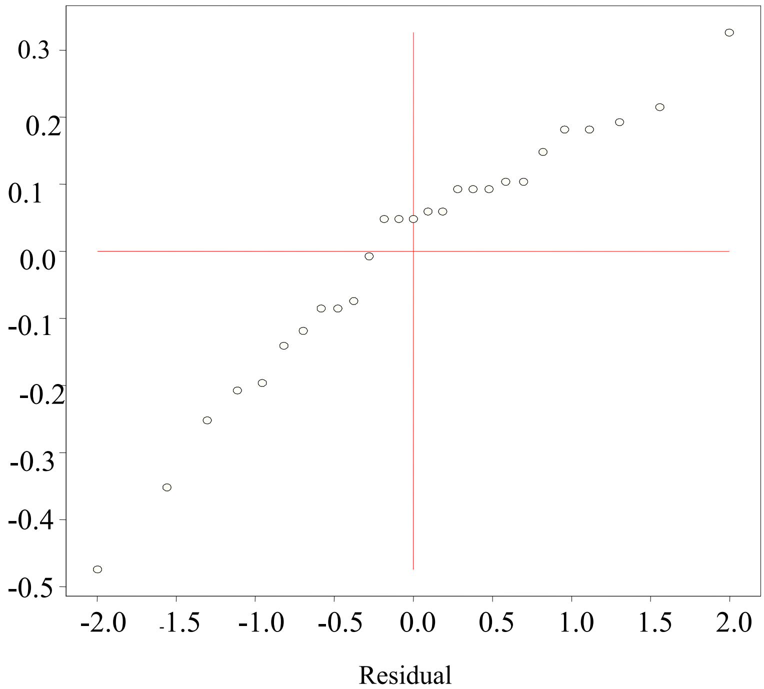

Fig. 3 Normal probability plot of residuals of model shown by Eq.6

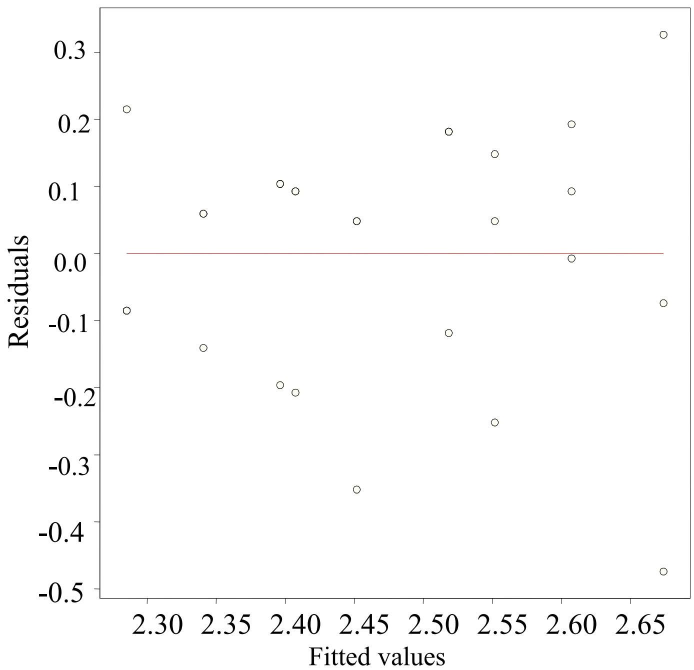

Fig. 4 Residuals versus fitted values for Eq.s 6-8

IV. Conclusions

The results obtained from this study indicated that aeration pretreatment technique reduced phytotoxicity and was the most effective method followed by settling in POME. However, settling was not effective in reducing phytotoxicity in CME within the 6-day period allowed to stand. pH neutralization increased POME and CME phytotoxicity compared to raw POME and CME without adjustment. Management plans for these effluent streams should consist of well designed pond system, metal tanks equipped with blowers for proper decomposition before disposal.

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