A pilot model for slaughterhouse wastewater treatment using Moringa oleifera seed husks, pods and extract followed by aeration

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A PILOT MODEL FOR SLAUGHTERHOUSE WASTEWATER TREATMENT USING *MORINGA OLEIFERA* SEED HUSKS, PODS AND EXTRACT FOLLOWED BY AERATION

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ABSTRACT

Untreated slaughterhouse wastewater contains high concentrations of organic matters, suspended solids, and pathogenic microorganisms which have destroying effects on the environment. Thus, slaughterhouse wastewater should be treated properly before discharging into the environment. A pilot model was installed that consisted of three treatment stages; bioadsorption, coagulation and aeration. The first stage used *Moringa oleifera* seed husks and pods as a bioadsorbent. The second stage used *M. oleifera* seeds extract as natural coagulant and followed by the third stage (aeration stage). *M. oleifera* seed husks and pods proved a good ability to remove total suspended solids (TSS), total bacterial count (TBC), total coliforms (TC) and *Escherichia coli*, without significant removal of organic matters. A significant increase in organic matters represented in chemical oxygen demand (COD), biological oxygen demand (BOD), total Kjeldahl nitrogen (TKN) and total phosphorus (TP) concentrations after the coagulation stage. The aeration stage succeeded to remove this occurred increase in the coagulation stage. The overall average removal percent after aeration stage were 95.68%, 95.89%, 70.15%, 82.98%m 83.91%, 90.49%, 90.58% and 88.66% for COD, BOD, TSS, TKN, TP, TBC, TC and *E. coli*, respectively. The obtained results indicated the suitability of using *M. oleifera* seed husks, pods, and extracts for slaughterhouse wastewater treatment.

Keywords: *Moringa oleifera*, slaughterhouse wastewater, physicochemical treatment, bioadsorption, natural coagulants.

INTRODUCTION

In developing countries, the increase in population growth has increased freshwater pollution due to the inadequate wastewater discharge. As a result, wastewater treatment has become a critical issue for societies development (Feng *et al*., 2009; Gopala Krishna *et al*., 2009). The meat processing industry uses approximately 62.0 million cubic meters of water per year. A small volume of this amount is a component of the final meat products, while the largest volume is a wastewater (Sroka *et al*., 2004). The meat processing industry consumes about 29% of that consumed by the agriculture sector (Gerbens-Leenes *et al*., 2013) and about 24% of the total freshwater consumed by the food and beverage industry (Bustillo-Lecompte and Mehrvar, 2015).

During the slaughtering and cleaning processes, abattoirs consume large amounts of water which in turn generate large amounts of wastewater (Kundu *et al*., 2013). Abattoir wastewater or slaughterhouse wastewater contains large amounts of liquid wastes and suspended solids in addition to odor generation (Gauri, 2006). The untreated effluents generated from slaughterhouses contain blood, urine, fats, and meat tissues which can reach the sewer system causing serious problems at wastewater treatment plants (Bello and Oyedemi, 2009). The main parts responsible for contamination are stomach, blood and intestinal mucus. The major dissolved pollutant in slaughterhouse wastewater is the blood which has the highest COD value of any other effluents. Aniebo *et al*., (2009) reported that if a single cow's carcass blood was allowed to discharge directly into the sewer system, the effluent load would be similar to the total sewage produced by fifty people on average day. United States Environmental Protection Agency (USEPA, 2004) classified slaughterhouse wastewater (SW) as one of the most dangerous and harmful types of industrial wastewater since the inappropriate disposal of SW causes groundwater pollution and rivers deoxygenation.

Slaughterhouse wastewater contains high chemical and biological oxygen demand, high concentrations of dry residues, fibers, high fat content, nitrogen and chlorides, detergents and disinfectants, heavy metals, colors, high suspended matters as well as pharmaceuticals for veterinary purposes. The presence of considerable amounts of proteins in slaughterhouse wastewater leads to off nasty smells and easily putrefying. Furthermore, slaughterhouse wastewater may contain pathogens and eggs of intestinal parasites and helminths such as Ascaris which originate primarily from blood, stomach and intestinal mucus (Tritt and Schuchardt, 1992; Sroka *et al*., 2004; Bustillo-Lecompte and Mehrvar, 2015).

In some developing countries, slaughterhouses often discharge wastewater into municipal sewer system without treatment or after insufficient primary treatment such as coagulation, flocculation and air floatation, which overloads wastewater treatment plants (Pan *et al*., 2014). Therefore, slaughterhouse wastewater requires proper treatment for safe and sustainable disposal into the environment.

There is a wide range of biological and physicochemical wastewater treatment technologies which have been used in removing different biological and chemical contaminants. Some authors have been preferred biological treatment methods which are simple and generate energy but generate large volumes of sludge and require high operational and maintenance cost (Liu *et al*., 2015). In contrast, other authors have been preferred the physicochemical treatment methods which are cost effective, easy to operate and energy saving but have poor performance in soluble organics' removal (Mahtab *et al*., 2009).

Among the various physicochemical methods, coagulation, and flocculation have been used for slaughterhouse wastewater treatment. Coagulation has been found to be cheap, easy to operate, great removal efficiency and energy saving. There are many organic (sodium alginate, polyacrylamide) and inorganic (sodium aluminate, aluminum sulfate, ferric chloride, polyaluminium sulfate, aluminum chloride, ferric sulfate) coagulants have been used for slaughterhouse wastewater treatment (Aguilar *et al*., 2005; Amuda and Alade, 2006; Mahtab *et al*., 2009).

Recently, many studies have focused on the use of natural coagulants in water and wastewater treatment as a viable alternative to chemical coagulants (Ndabigengesere and SubbaNarasiah, 1998). Moringa oleifera dry seeds are considered as one of the promising natural coagulants (Ferreira *et al*., 2008). *Moringa oleifera* belongs to the Moringaseae family which grows in tropical and arid places (Ndabigengesere and SubbaNarasiah, 1998). Different studies have shown the high effectivity of *Moringa oleifera* seeds as a natural biodegradable coagulant which are not toxic to animals or humans (Garcia-Fayos *et al*., 2016). *Moringa oleifera* seeds coagulant is efficient in removing microorganisms and turbidity from water (Ferreira *et al*., 2008), sludge conditioning, heavy metals' removal as well as in water softening (Ndabigengesere and SubbaNarasiah, 1998; Garcia-Fayos *et al*., 2016). In addition, *Moringa oleifera* seed husk is a waste generates during the preparation process of *M. oleifera* seeds coagulant (Kalavathy *et al*., 2010; Garcia-Fayos *et al*., 2016).

In the present study, a pilot model was evaluated for the treatment of three different types of slaughterhouse wastewater. In the pilot model, *Moringa oleifera* seed husks and pods (SHP) were used as a primary bioadsorbent followed by *Moringa oleifera* seeds powder as a natural coagulant and finally slaughterhouse wastewater was subjected to aeration.

MATERIAL AND METHODS

Preparation of *M. oleifera* seed husks and pods (bioadsorbent)

The seed husks and pods (SHP) preparation method was like as suggested by Garcia-Fayos *et al.* (2016). *M. oleifera* seeds were brought from a private company in Egypt with a harvesting date less than one month to prevent the reduction in natural coagulant (Katayon *et al*., 2006). SHP were manually removed then dried in a heater at 60°Cfor 24 h to remove humidity. The dried SHP was grinded uses a food processor (Kenwood™, UK). The grinded SHP was washed with distilled water since turbidity value of washed effluent was lower than 1 NTU. The SHP was again dried at 60°C for 24 h. Finally, the dried SHP was manually sieved to obtain a fraction size between 120 and 300 µm of diameter that used as a bioadsorbent.

Preparation of *M. oleifera* seeds powder (coagulant)

The husked *M. oleifera* seeds (the kernel) were crushed manually then mixed with ethanol (95%) at a ratio of 3:1 (ethanol volume/*M. oleifera* mass) and stirring for 30 min to reduce the oil content (Martín *et al*., 2010). The generated liquid was discarded, and the seeds were dried at 60°C for 24 h. Then, the dried seeds were grinded to produce a fine powder using a food processor (Kenwood™, UK). A 1M NaCl solution was prepared into which *M. oleifera* powder was mixed (5% w/v) and pH was adjusted at 7. Then the solution was vigorously stirred at room temperature for 30 min using a magnetic stirrer. After stirring, the mixture was filtered twice; first through a filter paper and second through a 0.45 µm glass filter (ThermoFisher Scientific™, UK). The filtrate was milky clear liquid and called *M. oleifera* stock solution which used as the coagulant. The stock solution was continuously stirred with a magnetic stirrer during the whole treatment process in order to avoid the settlement of proteins and other organic matters (Beltrán-Heredia and Sánchez-Martín, 2009). Jar test procedure was carried out to determine the optimum coagulation dose depending on turbidity removal.

Slaughterhouse wastewater collection

Slaughterhouse wastewater samples were collected from a domestic slaughterhouse in Cairo, Egypt. The processed animals are more than 55,000 heads per year. The volume of generated wastewater is about 60-190 m^3/d. A 100 L of slaughterhouse wastewater sample was collected daily in plastic containers then transferred rapidly to feed the pilot model.

Experimental procedure

Figure-1 describes the pilot model and summarizes the whole treatment process. Slaughterhouse wastewater samples were fed into the influent storage tank with a capacity of 150 L. The influent storage tank contained slow mixer (50 rpm) to prevent anaerobic conditions. The wastewater passed to the husk and pods tank (HPT) with a flow rate of 10 L/h, then passed under gravity to the coagulation tank. In the coagulation tank, *M. oleifera* stock solution was injected and continuously stirred for 90 min contact time followed by 15 min settling time. Wastewater then passed into the aeration tank with a retention time of three hours. The pilot model was continuously run for 15 days. The raw slaughterhouse wastewater pH was kept between 7.0 and 7.5 using 1N

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NaOH and 1N HCl solutions. Sludge was manually controlled through withdrawing from the coagulation tank.

Analytical methods

During the experiment period, a daily sample (sampling point 1) was collected from influent storage tank to characterize the quality of untreated slaughterhouse wastewater. Also, three samples were collected from the sampling points (Figure-1) every three hours to characterize the treatment performance of the pilot system. First sample was after HPT, second sample was after the coagulation tank, and third sample was after aeration tank (treated effluent). Before collecting the third sample, the aeration process was switched off for 10 min to allow settlement process. Some physicochemical parameters including pH, chemical oxygen demand (COD), biological oxygen demand (BOD), total suspended solids (TSS), total kjeldahl nitrogen (TKN) and total phosphorus (TP) were measured, as well as bacterial parameters including total bacterial count, total coli form and *Escherichia coli*. All parameters were measured according to the standard methods (APHA, 2010).

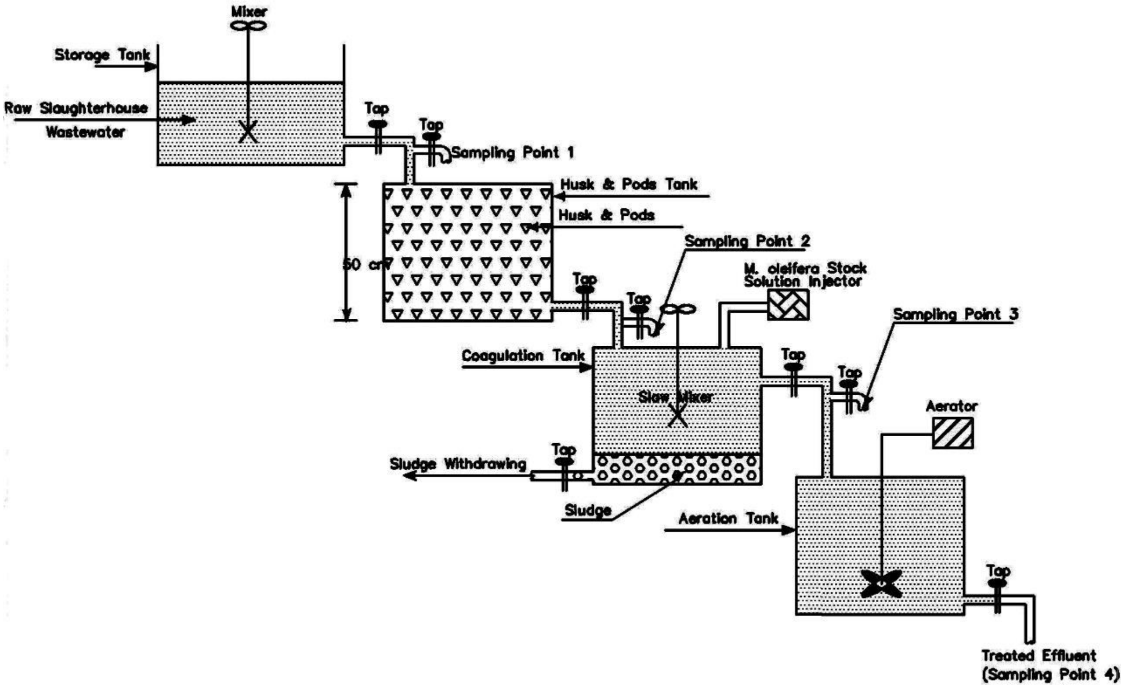

Figure-1. Schematic diagram of the pilot model.

RESULTS AND DISCUSSIONS

Slaughterhouse wastewater characterization

Table-1 shows the characteristics of raw (untreated) slaughterhouse wastewater. The raw slaughterhouse was neutral with average pH of 7.26. The effluent showed high concentrations of organic matters regarding COD, BOD, TKN and TP which owe to the presence of blood, fats, urine and soluble proteins (Al-Mutairi *et al*., 2004). Also, TSS, TBC, TC and *E. coli* concentrations and counts were high due to the presence of fecal matter and undigested food in slaughterhouse wastewater (Mahtab *et al.,* 2009). The obtained results of untreated slaughterhouse wastewater were the similar as reported results by Bustillo-Lecompte *et al*. (2016) since they reported high concentrations of organics, suspended solids and bacterial counts.

Table-1. Raw slaughterhouse wastewater characteristics.

Parameter	Unit	Average ± SD*	Minimum	Maximum
pH	-	7.26 ± 0.51	6.3	8.2
COD	mg/l	6742.4 ± 914.3	5380	8400
BOD	mgO_2/l	4201.5 ± 897.5	2978	6422
TSS	mg/l	1570.9 ± 549.4	730	2500
TKN	mg/l	517.8 ± 132.5	288	740
TP	mg/l	148.6 ± 63.8	52	301
TBC	cfu/ml	2953.3 ± 889.4	1700	4300
TC	cfu/100 ml	640 ± 72.89	750	980
E. coli	cfu/100 ml	393.2 ± 170.1	120	630

*SD: Standard deviation.

Bioadsorption efficiency of *M. oleifera* seed husks and pods

M. oleifera seed husks and pods (SHP) were used as a natural bioadsorbent material for the treatment of slaughterhouse wastewater. Table 2 summarizes the quality of slaughterhouse wastewater after passing through *M. oleifera* seed husks and pods. It was clear that SHP could remove the measured physicochemical and bacterial parameters with varied removal efficiencies. The organic materials represented in COD and BOD were slightly removed. The average removal percent was 16.75% and 14.16% for COD and BOD, respectively. In addition, TKN and TP showed almost the similar removal percent of 12.75% and 10.32%, respectively. There are different materials from organic origin such as seeds of different plants (Mataka *et al*., 2010), fruit wastes (Senthilkumaar *et al*., 2000), which showed adsorptive ability. Many researchers reported the ability of different parts of *M. oleifera* tree such as seeds, seed husks, pods, wood, and leaves as natural adsorbents to remove different types of pollutants from wastewater (Garcia-Fayos *et al*., 2016; Vunain and Biswick, 2018). These parts of *M. oleifera* tree were chemically modified with different techniques converting them into activated carbon which increased the removal efficiency of organic materials from wastewater. In the present study, *M. oleifera* seed husks and pods were not chemically modified which explained the low removal percent of organic materials. However, *M. oleifera* seed husks and pods showed remarkable removal of TSS, TBC, TC and *E. coli* with average removal percent of 41.19%, 50.85%, 51.79% and 52.22%, respectively. The reduction in TSS values occurred as a result of the adsorption and filtration nature of *M. oleifera* seed husks and pods which removed the solid particles from slaughterhouse wastewater (Barrera *et al*., 2012; Bustillo-Lecompte *et al*., 2013, 2014). Moreover, the decrease in TBC, TC and *E. coli* counts was due to the antibacterial activity of *M. oleifera* seed husks and pods. This antibacterial effect is due to the presence of some compounds such as polyphenols and tannins in *M. oleifera* which have been reported to possess antibacterial activities (Khosravi and Behzadi, 2006; Onsare *et al*., 2013; Isitua *et al*., 2016).

Table-2. Removal efficiencies of *M. oleifera* seed husks and pods bioadsorbent.

Parameter	Unit	Average ± SD*	Removal (%)		
			Minimum	Maximum	Average
COD	mg/l	5612.6 ± 883.6	9.85	28.5	16.75
BOD	mgO_2/l	3610.5 ± 802.4	10.2	19.7	14.16
TSS	mg/l	945.2 ± 401.1	30.0	51.5	41.19
TKN	mg/l	452.7 ± 119.1	8.1	21.5	12.75
TP	mg/l	134.2 ± 60.2	6.8	16.3	10.32
TBC	cfu/ml	1433.9 ± 424.2	40.0	64.2	50.85
TC	cfu/100 ml	416.4 ± 59.3	35.2	60.4	51.79
E. coli	cfu/100 ml	185.3 ± 82.8	42.0	67.6	52.22

*SD: Standard Deviation.

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Coagulation efficiency of *M. oleifera* seeds extract

Slaughterhouse wastewater passed into the coagulation tank after the bioadsorption process (bioadsorption tank). *M. oleifera* seeds extract was prepared and used as a coagulant for slaughterhouse wastewater treatment. The coagulant was injected into the coagulation tank and mixed slowly in order to allow the coagulation process to carry out. The settled sludge withdrew and disposed by sanitary landfilling. The clear slaughterhouse wastewater transferred into the aeration tank. Samples were collected from the clear slaughterhouse wastewater every 3 hours and examined for the measured physicochemical and bacterial parameters in order to study the efficiency of coagulation process (Table-3). The coagulation process led to average reduction in TSS concentration (81.75%) and reduction in bacterial counts of TBC (92.19%), TC (91.08%) and *E. coli* (86.04%). The coagulation ability of *M. oleifera* seeds is due to the presence of high amounts of proteins in the seeds which act as cationic polyelectrolytes once they added to water (Okuda *et al*., 2001; Ghebremichael *et al*., 2005). Moreover, the reduction in TSS values indicated the coagulation ability of *M. oleifera* seeds coagulant. The reduction in TSS led to a reduction in bacterial counts due to the settlement of attached bacteria with the settled particles in the generated sludge. The antibacterial effect of *M. oleifera* seed dissolved extractions was another factor that responsible for the reduction in TBC, TC and *E. coli* counts (Isitua *et al*., 2016). Although the obtained results proved the ability of *M. oleifera* seeds coagulant to reduce the bacterial counts in the treated slaughterhouse wastewater, the bacteria have not fully removed. Alo *et al*. (2012) and Arafat and Mohamed (2013) concluded the same behaviour of *M. oleifera* seeds coagulant for the removal of bacteria from wastewater. In contrast, an observed increase was carried out in concentrations of COD, BOD, TKN and TP after the coagulation process (Table-3 and Figure-2). The used coagulant of *M. oleifera* seeds has high concentrations of COD, large number of ions (NO_3^- and PO_4^{-3}) and high concentrations of orthophosphates (Ndabigengesere and Narasiah, 1998; Beltrán-Heredia and Sánchez-Martín, 2009). Thus, some of these organic matters didn't precipitate during the coagulation process and remained in the treated slaughterhouse wastewater. These organic matters also led to the increase in BOD concentrations. These obtained results were in comparable with the results reported by Adeniran *et al*. (2017). Shan *et al*. (2017) reported a dramatic increase in COD and BOD concentrations of wastewater after treatment with *M. oleifera* seed cake as natural coagulant, and they attributed this increase to the oil content and the remaining organic matter of *M. oleifera* seed cake. Some earlier studies reported the advantages of *M. oleifera* as a coagulant in wastewater treatment in comparison with the traditional coagulants such as alum and ferric chloride which are not suitable for use in developing countries (Beltrán-Heredia and Sánchez-Martín, 2009). These advantages can be classified into: (1) environmentally; *M. oleifera* coagulant is a natural extract that avoids the limitations related to alum usage, especially those concerned to aluminum intake causing Alzheimer (Ndabigengesere and Narasiah, 1998b; Flaten, 2001). (2) technologically; application of *M. oleifera* coagulant is much easier than traditional coagulants due to the stability in pH and stability in chloride and sulfate concentrations beside the smaller generation of sludge (Ndabigengesere and Narasiah, 1998a).

Table-3. Removal efficiencies of *M. oleifera* seeds coagulant.

Parameter	Unit	Average ± SD*	Removal (%)		
			Minimum	Maximum	Average
COD	mg/l	6719.4 ± 874.1	-36.36	-10.70	-20.37
BOD	mgO_2/l	4370.9 ± 865.8	-36.32	-13.28	-21.58
TSS	mg/l	170.3 ± 75.3	76.42	89.06	81.75
TKN	mg/l	520.8 ± 128.7	-22.22	-8.29	-15.61
TP	mg/l	155.3 ± 69.6	-23.91	-7.03	-16.31
TBC	cfu/ml	111.4 ± 36.2	90.98	93.69	92.19
TC	cfu/100 ml	36.86 ± 4.94	90.07	93.22	91.08
E. coli	cfu/100 ml	21.8 ± 4.34	74.62	93.37	86.04

*SD: Standard Deviation.

Efficiency of aeration process

The aeration stage was applied after coagulation stage in order to decrease the concentrations of COD, BOD, TKN and TP which increased during the coagulation stage (Table-3) depending on the ability of aerobic bacteria in removal of organics in the presence of oxygen. The aeration stage improved the quality of the treated slaughterhouse wastewater and showed great ability in removal of physicochemical and bacterial parameters as summarized in Table-4. COD, BOD, TKN,

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and TP removal percent increased significantly with removal averages of 95.68%, 95.89%, 82.98% and 83.91%, respectively. These removal percent were similar to the reported results of aerobic biological treatment of slaughterhouse wastewater (Pabón and Gélvez, 2009; Fongsatitkul *et al.*, 2011; Bustillo-Lecompte *et al.*, 2013, 2014). This increase in removal efficiencies proved the importance of aeration stage after the treatment process of slaughterhouse wastewater using *M. oleifera* seeds extract as natural coagulant. Chernicharo, (2006) and Bustillo-Lecompte and Mehrvar, (2015) clarified that aerobic treatment is commonly used for final nutrients' removal and final decontamination after using physicochemical techniques in wastewater treatment. In addition, the aerobic treatment is necessary if there was a need to remove nitrogen from wastewater (San José, 2004). The oxidation of organic matters in slaughterhouse wastewater is the main distinct mechanism of aeration process (Al-Mutairi, 2010). Additionally, the aeration process is creating conditions that favor the growth of floc forming bacteria more than filamentous bacteria especially in wastewater containing high levels of COD such as slaughterhouse wastewater (Al-Mutairi *et al.*, 2003, 2008).Regarding the decrease in bacterial counts (TBC, TC and *E. coli*), this may be due to the oxidation and decrease of organic matters present in treated effluent which are necessary to the growth, reproduction, and other metabolic activities of bacteria. The behaviour of the whole treatment process is represented in Figure-2.

Table-4. Removal efficiencies of aeration on slaughterhouse wastewater.

Parameter	Unit	Average ± SD*	Removal (%)		
			Minimum	Maximum	Average
COD	mg/l	288.0 ± 49.2	94.60	96.62	95.68
BOD	mgO_2/l	175.6 ± 27.8	94.67	97.27	95.89
TSS	mg/l	50.4 ± 22.3	63.73	79.80	70.15
TKN	mg/l	85.4 ± 20.3	74.53	89.60	82.98
TP	mg/l	24.06 ± 9.8	78.10	91.55	83.91
TBC	cfu/ml	9.73 ± 0.79	86.11	95.58	90.49
TC	cfu/100 ml	3.46 ± 0.83	87.09	93.75	90.58
E. coli	cfu/100 ml	2.4 ± 0.5	84.12	93.75	88.66

*SD: Standard Deviation.

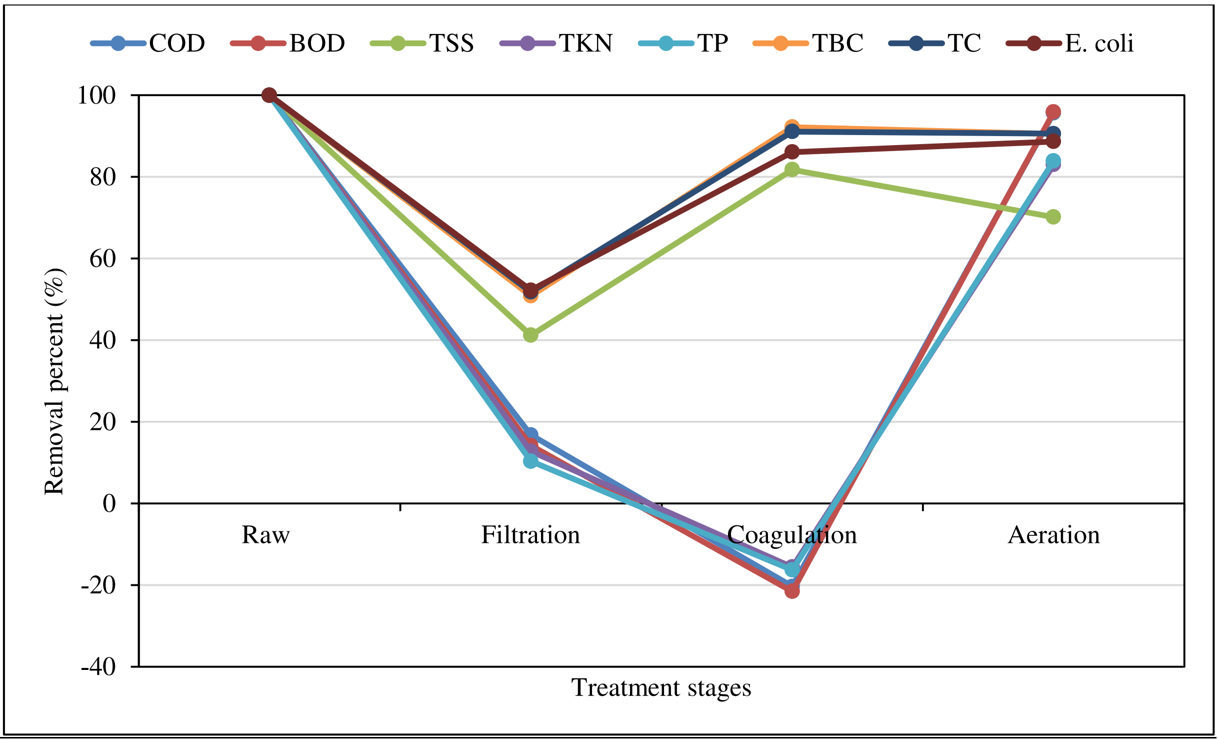

Figure-2. Behaviour of measured parameters during the whole treatment process.

CONCLUSIONS

In the present study, slaughterhouse wastewater was treated using *M. oleifera* seed husks and pods as a bioadsorbent followed by *M. oleifera* seeds extract as a natural coagulant and finally an aeration stage. From the obtained results it can be concluded that;

a) *M. oleifera* seed husks and pods effectively removed almost half concentrations and counts of TSS, TBC, TC and *E. coli*, while there was a very slight removal (10 - 16 %) of organic materials represented as COD, BOD, TKN and TP from slaughterhouse wastewater through adsorption and filtration mechanisms.

b) *M. oleifera* seeds extract that used as natural coagulant showed great ability in removal of TSS, TBC, TC and *E. coli*, while in contrast, it caused an increase in the concentrations of COD, BOD, TKN and TP due to the presence of high organics in *M. oleifera* coagulant.

c) Aeration was a necessary process after the coagulation stage which removed the organic matters from slaughterhouse wastewater which increased during the coagulation stage.

d) Application *M. oleifera* in the treatment of specific types of wastewater can decrease the quantities of used chemicals during the treatment process.

e) Application of *M. oleifera* in wastewater treatment can create a new cash crop for farmers as well as new jobs creation.

f) *M. oleifera* is a natural tree which can be used for the treatment of different types of wastewater.

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