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# Removal of Fecal Coliform from HFBR Effluent via Stabilization Pond as a Post Treatment

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**ABSTRACT**

This paper presents an evaluation of the performance for a pilot scale new biofilm reactor (HFBR) as a pre- treatment combined with an algal pond followed by duck weed pond as post-treatment for treating municipal waste water. These reactors were designed to further enhance the treatment efficiency and simplify the construction process in real scale, especially for the application in developing countries. HFBR system was operated at hydraulic and organic loading rate namely, $3.0m^3/m^2/d$ and$1006gCOD/m^2/day$ for period of over 9months.The combined system was able to remove 78%and 79% of unfiltered BOD and TSS, respectively with only 17mg/land 19mg/l remaining in the final effluent. Nutrient removal by the system was also satisfactory. Likewise, Fecal coliform were removed by 4 log with the final count ranged from $10^2$ to $10^3$ MPN/100ml. The study proved that the combination of HFBR with algal and duckweed pond give final effluent quality criteria meets the Egyptian permissible limits for safe disposal into the drains as well as the safe reuse in irrigation. The integrated system proved to be cost effective and represents a suitable treatment technology in rural and semi-urban areas in Egypt.

**Keywords:** Post- treatment, (HFBR), stabilization pond, pathogen removal, low cost, effluent reuse.

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## INTRODUCTION

Egypt has reached a stage of water poverty with an annual per capita share of water 663 $m^3$/y on average and expected to reduce to 582 $m^3$/y by 2025[1]. Egypt has reached stage of water poverty since not everyone has access to clean water supply 24 hours a day all the year. Remarkable reduction in the available clean fresh water make it financially burden to provide clean water at reasonable price. The dramatic deterioration in water quality is the inevitable result of dumping agriculture, industrial and municipal wastewater into Nile River and irrigation canals. Around 70% of the people in rural Egypt have some type of on-site septic tanks of house vaults [2], which represent the main source of water contamination. Direct disposal of untreated sewage into the ground water and emptying the septic tanks into the nearest waster body cause almost half of waterborne disease [3]. There is an urgent need for pollution prevention and better utilization of fresh water resources to meet the current and future projected needs of the rapidly growing population. Treated wastewater is considered as a non-conventional fresh water resource in the National Water Strategy of Egypt [4]. Reuse of the treated effluent is one of the solutions to overcome economic constrains and to improve cost recovery for sanitation services in Egypt [5].

Horizontal flow biofilm reactor represents low –cost and sustainable technology for sewage treatment, because of its low construction, operation and maintenance cost, small land requirement, low sludge production and a high quality of effluent produced from it [6, 7]. Despite of these advantages, the HFBR effluent still needs post- treatment to reduce residual Fecal Coliforms from the treated effluent to be the permissible limits for reuse.

Various technologies are available as post- treatment for pathogen removal, but most of these technologies are high in capital investment and generate more sludge with disposal problems [8,9]. Integrated Algal pond and duckweed pond as a post treatment system is an appropriate eco-technological system for countries with warm sunny weather and land availability. It is characterized by its low construction and maintenance cost, minimum energy requirements, high pathogen removal efficiency and reduction in sludge formation [10,11]. Recent studies proved that algal ponds are more efficient in fecal coliform removal than duckweed pond [12]. The decay of bacterial pathogen results from complex interactions of several factors such as sunlight radiation, high pH and high oxygen radicals especially in day time [13,14]. Also, other factors which such as, nutrients depletion, presence of anti-bacterial substances (algal toxins) produced by algae and sedimentation of attached Fecal Coliforms [15,16] play a role in pathogen removal. The main disadvantage of algal pond is the presence of algal biomass in the effluent which increases the TSS COD and BOD concentration in the pond effluent [17]. Algal biomass can be harvested and processed chemically and biologically to produce high value products such as bioacetone, biobutanol, biodiesel, and biomethane. Microalgae as feed stocks provide high densities of carbohydrates (typically comprising glucose units), triglycerides and free fatty acids that can be used to produce biofuels and biodiesel. It has been demonstrated that microalgae can be a promising feedstock and will play a vital role in the future production of clean and renewable energy [18].

AP effluent which is loaded with algal biomass could be passing through a stage with reduced illumination. This shading and reduced illumination is expected to cause the dying of algae with subsequent settling and disintegration. Duckweed ponds are covered by a floating mats of duckweed biomass which preventing light penetration into the pond. DP has been applied as a polishing treatment stage to remove nutrients from wastewater. The high growth rates of the macrophyte permits regular harvesting of bio-mass and hence nutrients are removed from the system. The duckweed bio-mass has an economic value because it can be applied as fodder fish [19,20]. The duckweed plant has been examined for nutrients recovery and biomass production using pre-treated sewage [21].

The main objective of this study is the development of low cost eco- biotechnological system for the treatment of domestic wastewater in rural areas and /or small communities. This system essentially consists of HFBR combined with Algal pond system especially for removal of bacterial pathogens followed by Duckweed pond for the removal of suspended solids and nutrients. The treatment technologies will focus on, reducing the pathogenic risk inherent to wastewater and facilitate the recovery of nutrient and water resources for reuse in irrigation purposes.

## MATERIAL AND METHODS

### Reactors and operational conditions

A continuous pilot-scale study of biological treatment system was designed, manufactured, installed and operated at municipal wastewater treatment plant under natural conditions. It consists of three units; HFBR was operated as a first stage of treatment. The second stage was Algal pond (AP)especially for removal of bacterial pathogens followed by Duckweed pond (DP) for the removal of suspended solids and nutrients (Figure1).

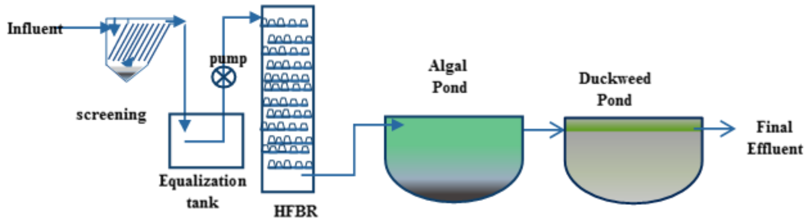



**Figure (1) A continuous pilot-scale of biological treatment system.**

### Design and operation of HFBR

HFBR is a simple and flexible design technology. The outer frame of the reactor is constructed from polyvinyl chloride (PVC) sheets. It consists of fifty-five horizontal (PVC) sheets, stacked one above the other and contained vertical frustums. The frustums increased the available biofilm plan surface area and provided for solids accumulation. Domestic wastewater pumped from a feed tank using a peristaltic pump into the top sheet of the reactor and flowed over and back along alternate sheets and down from sheet to sheet- sequentially through the reactor. The system was tested over 9 months at a hydraulic loading rate of 3.0 $m^3/m^2/d$ based on the top plan surface area of the system.

### Design and operation of Algal and duckweed ponds

Algal- pond with total surface area of 1 $m^2$ and 40 cm effective water depth was continuously fed with the HFBR effluent with HRTat5 days to remove bacterial pathogen. Algal pond was seeded with natural phytoplankton collected from the Nile River. Effluent of algal-based pond was clarified in a DP to remove the residual suspended solids and reduce the fecal coliform. The surface area of the DP was 1 $m^2$ while the water depth was 40 cm also the HRT was 5 days. Duckweed pond was seeded with fresh duckweed biomass (mixed culture of Lemna gibba and Lemna minor obtained from polluted water canal in Giza) at stocking density of 1100 $g/m^2$. Prior to stocking, the duckweed biomass was washed with tap water to remove detritus and impurities. Duckweed was harvested weekly and only one layer of duckweed mate was always left after harvesting.

### Analytical Techniques

### Analysis of Algal pond

The biomass of algae was monitored twice a week to determinate chlorophyll “a” content using spectrophotometric method according to [22]. and the changes in the community structure according to the key of the fresh water algae [23]. Total protein content was measured according to [24]. Total carbohydrate content was estimated as glucose using the spectrophotometric method described by [25].

### Analysis of duckweed biomass

Duckweed was harvested weekly and only one layer of duckweed mate was always left after harvesting. The harvested biomass of duckweed was drained; weighed to calculate the total harvested fresh biomass. Representative samples from the fresh duckweed were taken on weekly basis and dried in an oven at

70ºC to calculate the dry matter content. The daily growth and production of duckweed was calculated on fresh and dry bases.

**Determination of protein content**

A sample of dry matter of duckweed was taken and powdered in a tissue grinder. A representative sample of 0.1 g was taken and analyzed for the organic nitrogen using Kjeldahl method. The sample was digested by mercuric sulphate digestion method. Total ammonia of digested sample was determined titrimetrically upon distillation in the presence of strong sodium hydroxide. Protein content was calculated based on:
Protein (g/g) = organic N (g/g) × 6.25 [26].

**Determination of TP of dry biomass**

A representative sample of the dry duckweed was taken and digested using per-sulphate digestion method. After digestion the sample was neutralized and color was removed by charcoal followed by filtration on Whatman filter paper. The clear filtrate was analyzed for TP determination using vanado-molybdate method. The TP was calculated as mg P/g dry biomass.

**Sampling and physico-chemical and bacteriological characteristics**

Samples of raw wastewater and treated effluent of each unit were collected twice a week. Physico-chemical and micro-biological examination were carried out according to [22]. Fecal coliform was counted by poured plate technique using membrane Fecal coliform (m Fc media) and Ai direct media [22].

## RESULTS AND DISCUSSIONS

**Wastewater Characteristics**

Data recorded in Table (1), indicated that sewage COD ranged from218mgO$_2$/l to -465.mgO$_2$/l, with an average value of 332mgO$_2$/l. Soluble fraction of COD is about 51% of the total COD. Corresponding average BOD value of 184mgO$_2$/l.BOD$_5$/COD ratio were found to vary in the range of 0.46-0.56. Thus, the biodegradability of the domestic wastewaters is around 50% which is comparable to the published work of Egyptian municipal sewage in urban and semi-urban areas [27and21]. Average concentration of TSS and oil & grease were178mg/l and 45mg/l, respectively. Bacteriological examination of raw domestic wastewater gives geometric mean of Fecal Coliform was$2.1\times10^7$. The density of (F.C), presented as most probable numbers per 100 ml, ranged from $8.0\times10^5$to $5.7\times10^8$.These results are comparable to the published data of raw sewage in Egypt.

**Table 1: Characters of raw wastewater and treated effluents**

| parameters | Wastewater | HFBR effluent | A. P effluent | D.P Effluent | Disposal to agriculture drains* | Treated sewage for reuse** Grade B |
|---|---|---|---|---|---|---|
| pH-value | 7.6 | 7.7 ± 0.3 | 8 ± 0.3 | 7.3 ± 0.2 | 6-9 | |
| COD$_{tot}$ | 332 | 76 ± 8.2 | 146± 57 | 38 ± 9.6 | 80 | |
| COD$_{sol}$ | 162 | 34 ± 7.7 | 75 ± 32 | 18±6 | | |
| BOD | 184 | 38 ± 7.8 | 77± 31 | 17±7 | 60 | 60 |
| TSS | 178 | 26 ± 11 | 68± 28 | 19±5.8 | 50 | 50 |
| T.P | 2.2 | 1.1 ± 0.2 | 0.85± 0.9 | 0±0.8 | - | |
| T.K.N | 52 | 18 ± 11 | 11± 13 | 8.6±10 | - | |
| Ammonia | 24 | 14 ± 5.6 | 3.1 ± 6 | 1.9±3.9 | - | |
| Oil & Grease | 45 | 8 ± 5 | | | 10 | |
| Fecal coliform | $2.1\times10^7$ | $2.8\times10^5\pm 4.7\times10^5$ | $3.1\times10^3\pm 4.6\times10^3$ | $1.3\times10^3\pm 1.2\times10^3$ | 5000 | 5000 |

*** Egyptian law 48 for 1982. **Modified Ministerial Decree 44 for 2000.**

**Performance of HFBR**

The results recorded in Table (1), clearly proved the high performance of HFBR in the removal of carbon and nitrogen. The removal efficiency of COD, BOD, TSS and Oil & Grease were 79, 82, 82 and 83% (Figure 7) respectively with average effluent concentration were 76, 38, 26 and 8 mg/l (Figure 2 & 3), respectively. Also, the residual concentration of organic nitrogen was 4 mg/l. Mean residual phosphorous and ammonia concentration were 1.1mg/l, and14mg/l, respectively. Physico-chemical characteristics of HFBR effluent meets disposal limits to the drains and reuse standard for irrigation or group B category. Residual Fecal Coliform were extremely variable, ranging from 6 × $10^3$ to 1.7× $10^6$ MPN-index/100ml with an average value 2.8×$10^5$MPN-index/100mlis the only constrain for safe disposal or reuse (category B).

**Algal pond performance:**

Effluent quality of the algal pond recorded in Table (1) and illustrated in Figures (2 & 3). The results showed that pH range between 7.8 -8.8. Residual concentration of COD and TSS has been increased to average values of 146mg/l and 68mg/l, respectively, these concentrations were increased by more than 100% from the residual concentration of the HFBR effluent. This was attributed to the presence of algal biomass in the effluent. Similar results have been reported by [28&29 and 7].

Bacteriological analysis proved that AP has high efficiency in fecal coliform reduction; it could remove 2 logs of bacterial count. The removal percentage reached 98.6% with average residual concentration 3.1×$10^3$ MPN-index/100ml (Figure 4).

It was confirmed that it is not necessary to reach a pH value between 9.0 and 9.3 to cause rapid pathogen decays suggested by other researchers [30,31; 32]. Rapid pathogen decay took place due to intensity of the solar radiation and high oxygen concentrations that able to damage fecal bacteria indicator [33]. The high removal of Fecal Coliform bacteria in AP unit could also be a result of receiving direct sunlight which leads to prolonged ultraviolet exposure (UV) radiation [34].

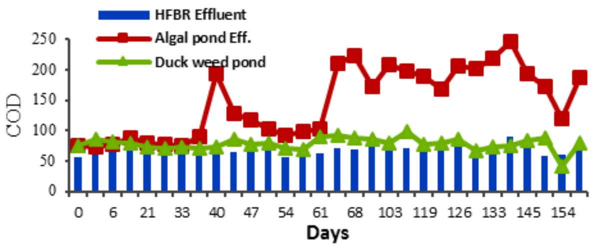



Figure (2) The residual concentration of COD

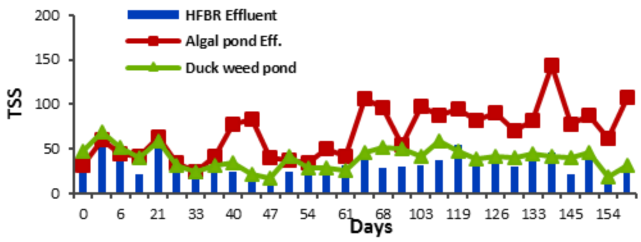



Figure 3 The residual concentration of TSS

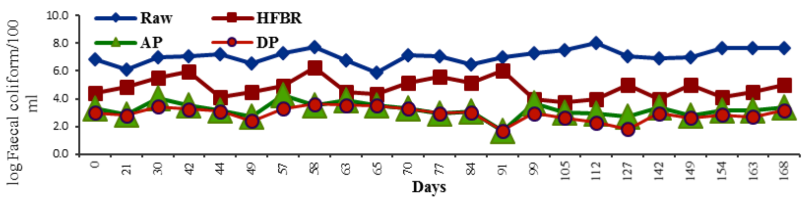



Figure 4 Fecal Coliform concentration

**Algal community structure:**

The distribution pattern of algae in the treatment plant throughout the study period recorded in Table (2). The AP is an open pond which is difficult to control the culture conditions, thus only few microalgae species can be successfully dominant. At the beginning of operation, the algal community structure showed that, it contains algal species belonging to four algal group namely *Chlorophyta, Euglenophyta, Cyanophyta and Bacillariophyta*. During the experimental run, changes in diversity and redundancy of algal population took place. The more sensitive algal species disappeared completely, while the tolerant forms resisted the condition and increased in numbers. During the studying period *Euglenophyta* represent the most abundant group present in good number followed by chlorophyte group. After two months of the system operation, the most dominant algal species were *Scenedesmusquadricauda, Euglena sanguinea, Phacuslongicauda,* while the rare species were *Merismopediaglauca and Nitzschialinearis*; and the other species not detected, these results continuous till the end of the study. The variation in algal community structure may be due to variation in temperature in different season in addition to the ability of certain species to grow fast and be dominant rather than another species.

**Table 2: Community structure and species dominancy**

| Algal taxa | Initial flora | Algal flora at operation steady state |
|---|---|---|
| **Chlorophyta** | | |
| *Scenedesmusquadricauda.* | + | ++ |
| *S. obliquus* | + | - |
| *StaurastrumParadoxum* | + | - |
| **Euglenophyta** | | |
| *Chllomonasparameculum* | ± | - |
| *Euglena sanguine* | ± | +++ |
| *Phacuslongicauda* | ± | ++ |
| **Cyanophyta** | | |
| *Chroococcuslimneticus .* | ± | - |
| *Microcystisflos-aquae* | + | - |
| *Merismopediaglauca* | + | ± |
| **Bacillariophyta** | | |
| *Cyclotella comta* | ++++ | - |
| *Gomphonemaparvulum* | ++ | ± |
| *Melosiragranulata* | + | |
| *Naviculaconfervaceae* | + | ± |
| *Nitzschialinearis* | ++ | ± |
| *Diatomaelongatum* | +++ | - |
| *Synedra ulna* | + | - |
| *Nitzschiaholsatica* | ± | - |
| ++++: Dominant; +++: Plenty; ++: Many; +: Appreciable; ±: Rare; –: not detected. | | |

**Growth measurement of Algal biomass**

Chlorophyll (a) was measured in order to evaluate the growth rate of the community structure in AP since this parameter is widely recognized to be directly correlated with algal biomass density [35]. Chlorophyll (a) concentrations increase gradually from 468 to 2987 with an average of 1319 µg/l (Figure5), these concentrations can be correlated with the distribution pattern of algal population. The increase in chlorophyll (a) in the last two months (July and August) is due to increasing in temperature since algal productivity increased with increasing pond temperature [36]. On the other hand, positive correlation between protein, carbohydrate and chlorophyll “a” content of algal biomass took place (Fig.6). However, the maximum bio-mass (chl.(a) 2.987mg /L) was equivalent to 39.5&12.9 mg/gm of protein and carbohydrate of dry weight, respectively.

**Performance of Duckweed pond**

The effluent quality of the DP showed significant reduction in the algal biomass represented by TSS, COD and BOD concentrations (Table 1 and Figures (2 &3). The COD, TSS and BOD concentrations were reduced to 38 and 19 mg/l and 17mg/l, respectively with corresponding percentage removal values of44%,45% and 44%, respectively from AP effluent (Figure 7).

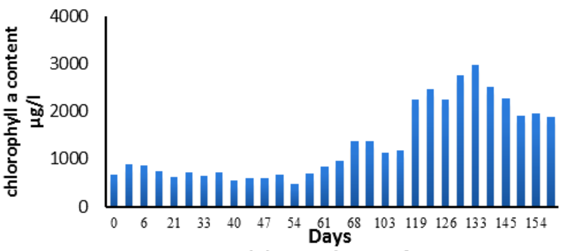



Figure (5) Growth rate of AP

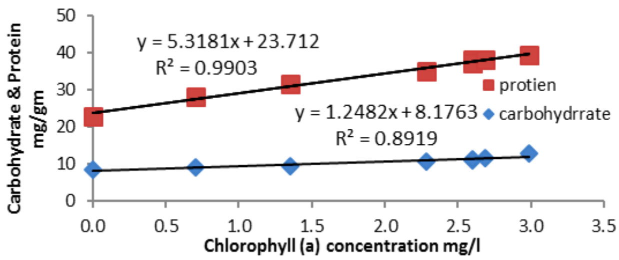
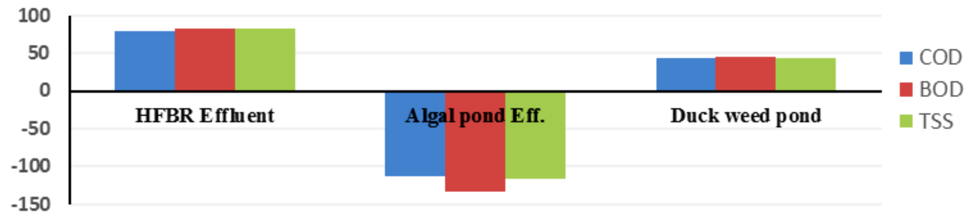



Figure (6) Relation- ship between chlorophyll (a) content and carbohydrate & protein concentration

For Fecal coliform, the DP has limited efficiency and the count was not significantly reduced. The count of FC was reduced by less than one log Figure 4. [21], reported that removal of Fecal coliform in duckweed ponds is more efficient in the warm season, also duckweed reduced Fecal coliform count via nutrients recovery and adsorption of the coliform during duckweed harvesting.

Figure ( 7 ) Performance of the diffrent treatment units




**Nutrients removal in duckweed ponds**

The results of nutrients removal in the duckweed pond are depicted in Table (3). The results show ability of duckweed pond to remove 68% of the total nitrogen in the influent which is corresponding to daily removal rate of 7.5 kg N/ha.d on average, which is higher than the reported value (5.5 kg N/ha.d) of *Lemna gibba* grown on pre-treated sewage [21]. The plant uptake of nitrogen was 3.90 kg N/ha.d which represents 52% of the total nitrogen removal while 48% was attributed to sedimentation, nitrification/denitrification and other processes. The higher removal of N in the DP here is mostly attributed to the process of di-nitrification since the pond receive algal pond effluent with considerable amounts of oxidized nitrogen (3.3mgN/l). On the other hand the results of a full-scale duckweed-covered sewage lagoon [37], revealed that 44% from the TN input has been recovered by the duckweed which corresponds to 2.6 kgN/ha.d, which is far below the value of this study. The duckweed of this research achieved phosphorus uptake rate of 70 mg which is comparable to the maximum value (74 mg P/m$^2$. d) reported by [38].

**Characterization of harvested Duckweed**

Results of the growth performance of duckweed and daily production rate of the biomass were shown in Table (4). The results show average daily fresh biomass production of 299 g/m$^2$.d which is equivalent to 3.0ton/ha.d. This result is comparable to the reported value (2.6-2.8 ton/ha.d) of duckweed *Lemna gibba* grown on pre-treated sewage [21].The corresponding dry matter production is 10.6 g/m$^2$.d which equivalent to 106 kg/ha.d. The dry matter content of duckweed varied between 3.0 to 4.2% which is relatively lower than the range of 5.3-6.3% by [26].

The results of this study show that protein content of dry matter was 23.1% on average and the corresponding range was 19.3-28.0%. This protein content is similar to the range of protein content (19.8-25.7%) of duckweed grown on pre-treated sewage [21], while it is higher than the range of protein content (15.8-28%) of duckweed species grown on sewage [39]. It is also comparable to 26.9±3.7% reported for *Lemna gibba* grown on sewage [26].

Also, T P content of the dry biomass show and average value of 0.64% (0.56-0.72%) which is little lower than the range of 0.68-0.90% of *Lemna gibba* grown on pre-treated sewage but higher than the range (0.48-

0.88%) reported by others [37; 39].TP content of dry duckweed in this study is similar to the reported value of *Lemna gibba* grown on sewage during spring [26].

**Table 3: Nitrogen and P recovery rates in duckweed pond**

| Parameter | Unit | Average ± std |
|---|---|---|
| **TN removal** | % of AP Effluent | 68±42 |
| **TN removal** | kg N/ha.d | 7.5±2.6 |
| **N recovery** | kg N/ha.d | 3.9±0.6 |
| **N recovery** | % of TN removal | 52±23 |
| **P recovery** | mg P/$m^2$.d | 70±15 |

**Table 4: Daily production of duckweed biomass and its characteristics**

| Item | Unit | Average ±std | Minimum | Maximum |
|---|---|---|---|---|
| **Fresh duckweed** | g /$m^2$.day | 299±62 | 217 | 369 |
| | Ton/ha.day | 3.0±0.6 | 2.2 | 3.7 |
| **Dry duckweed** | g /$m^2$.day | 10.6±2.3 | 8.0 | 14.0 |
| | Kg/ha.day | 106±23 | 80 | 140 |
| **Dry matter content** | % | 3.7±0.5 | 3.0 | 4.2 |
| **Organic matter content** | % | 84.5±1.7 | 82.8 | 86.6 |
| **Ash** | % | 15.5±1.7 | 13.4 | 17.2 |
| **Nitrogen content** | % of dry matter | 3.70±0.54 | 3.08 | 4.48 |
| **Protein content** | % of dry matter | 23.1±3.4 | 19.3 | 28.0 |
| **P content** | % of dry matter | 0.64±0.08 | 0.56 | 0.72 |

### Performance of the integrated system

The results obtained showed that the integrated system produced high quality effluent with COD, BOD and TSS residual concentration values of 38mg/l, 17mg/l and 19 mg/l, respectively remaining in the final effluent. Residual Fecal Coliform were extremely variable, ranging from $10^2$ to $10^3$ MPN/100ml with average value of $1.2\times10^3$MPN/100ml in the final effluent.

## CONCLUSIONS

From the available data it can concluded that the integrated system (HFBR+AP+DP), produce treated effluent with high quality which compiles with Egyptian legislation for safe disposal into the agricultural drains and can be reuse in irrigation of category B according to the Egyptian code of sewage reuse in agriculture. The proposed system has good performance, simple operation, low construction cost and less energy consumption. It is good alternative for the conventional treatment system, it is highly recommended for small communities and urban areas. Additional benefit is the production of macrophyte (duckweed) biomass with high protein content (23%) which makes it good alternative for animal fodder and high cost feed ingredients.

## ACKNOWLEDGMENT

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