Enhanced Treatment of Contaminated Domestic Wastewater Using Bacterial Consortium Biofilm

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Abstract

The study aimed to investigate decontamination of domestic wastewater using bacterial consortium biofilm consisted of four strains {***Pseudomonas stutzeri* M15-10-3 (PS), *Bacillus* sp. OU-40 (Rz6), *Bacillus amyloliquefaciens* T004 (S1)** and ***Bacillus cereus* OPP5 (Rz7)**}. Bacterial mixed culture fixed on gravels was tested continuously at different flow rates (100, 150 and 200 ml/h) for 5 working hours where samples were collected on hourly interval. Raw wastewater samples were collected from the drainage network. Wastewater quality parameters including pH, temperature, dissolved oxygen (DO: 5-9 mg/l), total suspended solids (TSS: 140-155 mg/l)), total dissolved solids (TDS: 390-420 mg/l), biochemical oxygen demand (BOD: 111-120 mg/l), chemical oxygen demand (COD: 350-448 mg/l), fat, oil and grease (FOG: 34-38 mg/l), bacterial total viable count (TVC: 4.00 x 10^6 and 16.0 x 10^7), total coliform (TC) and fecal coliform (FC) were determined before and after treatment and the removal efficiencies (REs) were calculated. As a general trend, the RE of all the tested parameters increased with increasing the exposure time and decreased with increasing the flow rate. The highest achieved removal efficiencies by the proposed biofilm system recorded 86.0, 84.0, 83.7, 98.5, 27, 99.8, 100 and 98.8% for TSS, BOD, COD, FOG, TDS, TVC, TC and FC after 5 running h at the lowest tested flow rate (100 ml/h) except for FOG (150 ml/l). Treatment using the biofilm system has led to decrease of all the tested pollutants to much lower levels than the maximum permissible limits (MPL) for safe discharge into open environments. Results of the present study confirmed that the proposed biofilm system using a composite culture is highly active; very promising; renewable and recommended cheap biotechnology for the treatment of wide range of contaminated domestic wastewater.

Key Words: Biofilm; Bacteria, Consortium, Contamination; Domestic Wastewater; Treatment

INTRODUCTION

Wastewater is any water that has been adversely affected in quality by anthropogenic influence. It comprises liquid waste discharged by domestic residences, commercial properties, industry, and/or agriculture and can encompass a wide range of potential contaminants and concentrations. Sewage, also known as black water, is correctly the subset of wastewater that is contaminated with feces or urine, but is often used to mean any wastewater **(McGraw-Hill Encyclopedia of Science** and **Technology 2009; Environmental Protection Agency, EPA, 2011)**. Human wastes can be a serious health hazard, as it is a good vector for both viral and bacterial diseases. A major accomplishment of human civilization has been the reduction of disease transmission via human waste through hygiene practices and sanitation, including the development of sewage systems and plumbing **(Cilimburg *et al.*, 2000)**.

Sewage composition varies widely but may contain more than 95% water with pathogens (bacteria, viruses, parasitic worms, protests and helminthes) **(Griffin *et al.*, 1999; Shannon *et al.*, 2007; Ziska** and **Runion, 2007; Symonds *et al.*, 2011; Widerström *et al.*, 2014)** and non-pathogenic bacteria (>100,000 CFU/ml for sewage). Chemically, sewage water is a

complex matrix, with many distinctive chemical characteristics with high conductivity (due to high dissolved solids), high alkalinity and pH typically ranging between 7 and 8. Trihalomethanes are also likely to be present as a result of past disinfection. Chemical contaminants include solid organic particles (faeces, hairs, food, vomit, paper fibres, plant material, humus, etc.), soluble organics (urea, fruit sugars, soluble proteins, drugs, pharmaceuticals,etc.), inorganics (sand, grit, metal particles, ceramics, etc.), soluble inorganics (ammonia, road-salt, sea-salt, cyanide, hydrogen sulfide, thiocyanates, thiosulfates, etc.), animals (protozoa, insects, arthropods, small fish, etc.), macro-solids (sanitary napkins, nappies/diapers, condoms, needles, children's toys, dead animals or plants, body parts,etc.), gases (hydrogen sulfide, carbon dioxide, methane, etc.) and finally toxins (pesticides, poisons, herbicides, etc.) **(Tchobanoglous *et al.*, 2003; Shannon *et al.*, 2007; Melosi, 2010; PUB-Singapore National Water Agency, 2011).**

Sewage with the included physical, chemical and biological contaminants directly and indirectly impacts the wastewater treatment plants efficiency as well as the receiving aquatic environments. For example, the environmental impacts of wastewater temperature, pH, odor, flow, turbidity, color, inorganic ions **(Thomas *et al.*, 2003)**, organic matter and biological contaminants **(Tobin *et al.*, 1981; Harris *et al.*, 1990, Taylor *et al.*, 1997; Ahsan *et al.*, 2006; Ziska** and **Runion, 2007; Conejero, *et al.*, 2011)** were discussed earlier.

Sewage or domestic wastewater treatment is the physical, chemical, and biological removal processes of contaminants from wastewater and household sewage in order to produce treated effluent and solid waste or sludge suitable for discharge or reuse back into the environment. Although conventional sewage treatment involves primary, secondary and tertiary treatment stages, secondary (biological) treatment considered the main process where it removes dissolved and suspended biological matter and is typically performed by indigenous, water-borne microorganisms in a managed habitat **(SriuNaik** and **PydiSetty, 2011;Tilley, 2011)**. In that stage, bacteria and protozoa consume biodegradable soluble organic contaminants (e.g. sugars, fats, organic short-chain carbon molecules, etc.) and bind much of the less soluble fractions into floc. If domestic sewage is mixed with sources of industrial wastewater it will often require specialized treatment processes **(Ting *et al.*, 2013; Sharma** and **Sanghi, 2012)**.

Secondary treatment systems may be designed as fixed-film or suspended growth secondary treatment systems (activated sludge and surface aerated basins) **(Henze *et al.*, 2008; Benidickson, 2011)**. In contrary with their planktonic (free-living) counterparts, bacteria within biofilms are remarkably resistant to many natural and artificial factors including traditional antimicrobial agents **(Vega *et al.*, 2014; França *et al.*, 2016),** disinfectants **(Marvasi *et al.*, 2015)** since they are protected by extracellular polymers. In the industrial and medical sectors microbial biofilms represent a major challenge. Therefore, serious attempts to monitor **(Blanco *et al.*, 2011; Brooke *et al.*, 2014)**, control and/or prevent their development were investigated using different agents such as the fungicides pyrimethanil and carvacrol **(Abelho *et al.*, 2015; Gharbi *et al.*, 2015)** or using very promising biofilm dispersal agents such as nitric oxide (NO) encapsulated within a hydrogel composed of cellulose nanocrystals (CNC) **(Marvasi *et al.*, 2015).** Moreover, different enzymes such as pectin methylesterase **(Torres *et al.*, 2011)**, 5kDa peptide fraction (5-HCC) of the cytosol from sea-cucumber ***Holothuria tubulosa*** coelomocytes **(Schillaci *et al.*, 2013)** and Paracentrin 1 from the 5-kDa peptide fraction from the coelomocyte cytosol of the sea-urchin ***Paracentrotus lividus*** **(Schillaci *et al.*, 2014)** were documented as active biological agents controlling the growth of harmful biofilms.

However, biofilms are beneficial in other applications such as wastewater systems where they resist serious contaminants such as heavy metals even at high concentrations **(Golby *et al.*, 2014)**. Also they are the most reactive component in natural aquatic environments where they perform indispensible roles in cycling of essential elements such as carbon and nitrogen as well as biodegradation of pollutant organic wastes **(Ylla *et al.*, 2014)**. Microbial N-cycling nirS and nirK (denitrification through the conversion of NO_2 to NO), nifH (N_2 fixation), anammox (anaerobic ammonium oxidation), and amoA (aerobic ammonia oxidation, both bacterial and archaeal) genes were found in epilithic biofilms of a set of high-altitude oligotrophic lakes in the **Pyrenees, Spain**. This metabolically diverse epilithic biofilm community has the potential to carry out an active role in the biogeochemical nitrogen cycling of high altitude ecosystems **(Vila-Costa *et al.*, 2014)**. Moreover, thirteen marine bacterial biofilm strains have been isolated from different inert surfaces immersed in the Mediterranean Sea at the **Bay** of **Toulon** (**France**). They were belonging to 8 different genera and 12 different species. ***Shewanella*** sp. and ***Pseudoalteromonas*** sp. were the most predominant genera recovered and 2 novel bacterial species named ***Persicivirga mediterranea*** isolated for the first time from the **Mediterranean Sea (Brian-Jaisson *et al.*, 2013)**. Genetic engineering of biofilm strains can remarkably enhance their resistance and ability to degrade environmental contaminants **(Perni *et al.*, 2013; Noack-Schönmann *et al.*, 2014)**. Therefore, the present study aimed to manipulate the marvellous ability of multispecies bacterial biofim for decontamination of domestic wastewater. Bacterial consortium biofilm consisted of four strains {***Pseudomonas stutzeri*** **M15-10-3 (PS),** ***Bacillus*** **sp. OU-40 (Rz6),** ***Bacillus amyloliquefaciens*** **T004 (S1)** and ***Bacillus cereus*** **OPP5 (Rz7)**}.

MATERIALS AND METHODS

Sampling

Domestic wastewater samples were collected from the domestic wastewater drainage network in Jeddah City, Saudi Arabia during the course of the study. They were collected in pre-sterilized bottles where temperature and pH were measured at the collection points. Sewage samples were subjected to physicochemical as well as microbiological characterization to define their pollution strength and selecting the best treatment technology. In addition, post-treatment characterization took place in order to evaluate treatment efficiency.

Microorganisms

Seven domestic wastewater indigenous isolates as well as exogenous bacteria were tested during the present study to select the most promising. Among those four strains were selected and identified using molecular techniques **(El-Bestawy *et al.*, 2014)** and used in the bioremediation assays during the present study. Two are indigenous species isolated from the contaminated wastewater samples {***Bacillus*** **sp. OU-40 (Rz6)** and ***Bacillus cereus*** **OPP5 (Rz7)}** and two are exogenous species **{*Pseudomonas stutzeri* M15-10-3 (PS)** and ***Bacillus amyloliquefaciens*** **T004 (S1)**. The four selected bacterial species were investigated as mixture fixed on gravels in continuous bioassay at different flow rates (100, 150 and 200 ml/h) for 5 working hours where samples were collected on hourly interval. S1 and PS were originally isolated from heavily polluted media (wastewater and environments) and previously exhibited superior pollution decontaminating ability **(El-Bestawy *et al.*, 2013** and **2014)**.

Media preparation and culturing conditions

Nutrient broth and agar (NB & NA) were used as a general medium for enumeration, purification, transferring and preservation of viable bacteria in sewage samples. NA medium contained (g/l) peptic digest of animal tissue, 5.0; yeast extract, 1.5; beef extract, 1.5; sodium

chloride, 5.0 and agar, 15.0 (in case of NA). NB and NA media ingredients were supplied by (Himedia, India). Medium pH was adjusted to 7.2, sterilized by autoclaving at 121ºC for 20 min and used freshly for growth experiments as well as biodegradation assays.

Total and fecal coliform bacteria (TC & FC) were determined using Chapman TTC Agar (Tergitol® 7 Agar). It is a coliform selective dehydrated medium (Lactose TTC Sodium Heptadecylsulfate Agar) supplied by (Scharlau, Spain). It was sterilized as mentioned above and used freshly for coliform counting. After culturing the selected bacterial species were incubated at 37°C for 24 hours.

Bacterial counting

Changes in the total heterotrophic bacterial viable counts (TBVC) during wastewater treatment were determined using pour plate technique of the standard plate count method **(Clesceri *et al.*, 1999)**. After sequential dilutions of the raw and treated samples, specific volume (100, 500 or 1000 µL) from the final dilution were cultured in NA medium and incubated at 37ºC for 24 hours. Colony forming units (CFU) of the total viable bacterial counts (TVC) were recorded and averages were calculated.

The Coliform group consists of several facultative anaerobic, gram negative, non-spore forming rod shaped bacterial genera belonging to the family Enterobacteriaceae. Membrane filters technique (MF) of the standard coliform count test is highly reproducible and yields numerical results more rapidly than the multiple tube fermentation procedure, thus, it was used during the present study. Total coliform bacteria retained on polycarbonate bacterial membranes (22 µm) after filtration of specific volume of the sewage water sample were grown on Chapman TTC Agar medium containing lactose and shown as red colonies with a metallic (golden) sheen after 24 hours incubation at 37ºC. The same technique was performed for determination of fecal coliform bacteria after incubation for 24 h at 45ºC. Fecal coliform bacteria took various shades of blue. Non fecal coliform colonies were grey to cream. All samples were analyzed in triplicates.

Bioremediation bioassays using fixed bacteria (biofilm)

Indigenous as well as exogenous bacteria were tested visually for bioremediation of the raw municipal wastewater. They were inoculated individually in 250 ml flasks containing wastewater effluent and incubated at 37°C under 100 rpm agitation speed. Visual observations (degree of water clearance) were taken daily for a week according to which four promising isolates for bioremediation process were selected.

Biofilm system development

Based on the results of the free living bioassay **(El-Bestawy *et al.*, 2014)** and the removal efficiency of the investigated parameters the mixed culture (four strains) was selected since it showed broad and highest degradation activity and capability for remediating the contaminants in the sewage effluent compared to the individual strains. Prior to remediation experiment, liquid mixed culture was prepared in NB medium and incubated overnight.

Two cylindrical plastic columns (30 x 7 cm) were sealed at the bottom by a porous net (d<1mm) and supplied with a flow controller (tap) at the outlet **(Fig. 1)**. They were sterilized by immersing in 75% ethyl alcohol overnight, rinsed twice with absolute ethanol, and five times with sterile distilled water, and then dried in a sterile condition. Gravels (≈5 mm in diameter) were used as supporting material after thorough washing, rinsing and sterilization 3 times at 121°C for 1 hr. Each cylinder was packed with sterile gravels up to 80% of their

height leaving the top 20% free. After packing, one column was used as a control where only wastewater was supplied during the treatment stage, while the other column was inoculated with 400 ml dense overnight mixed liquid culture (6.0 x 10^8 CFU/ml) and left 10 days to allow bacterial cells adhesion forming the biofilm. The two columns were connected with an up flow air supply, which was adjusted to operate alternately for 1 h and pause for 2 h.

Figure 1. The biofilm system

The seeded column was left as a batch culture at pH 7, temperature ranged between 20 and 25 °C (room temperature). After 10 days, a sample from the biofilm column was collected every 24 hr, serially diluted (up to 10^{-8}) and 100 µl of the appropriate dilution was cultured on NA and incubated for 24 hr at 37 °C. Bacterial plate counts were recorded every day till constant count was obtained for two consecutive days.

Raw samples were treated using the biofilm at different flow rates (100, 150 and 200 ml/h). At each flow rate, samples were collected from both the biofilm and bacteria-free (control) columns at 30 min interval for 2.5 hours. After treatment, all samples were characterized for the same parameters as for the raw wastewater and the efficiency of the treatment using the proposed biofilm for these contaminants was calculated.

Characterization of the Raw and Treated Sewage Effluent

Wastewater was characterized before and after the proposed treatment. Characterization of the wastewater included its pH, temperature, dissolved oxygen content (DO), total suspended solids (TSS), total dissolved solids (TDS), biochemical oxygen demand (BOD), chemical oxygen demand (COD), fat, oil and grease (FOG), total viable count of bacteria (TVC), count of total and fecal coliform bacteria (TC & FC) all of which were determined using the standard techniques described in Standard Methods for the Examination of Water and Wastewater **(Clesceri *et al.*, 1999)**. After treatment the selected parameters were analyzed to determine their residual levels at each exposure time. Removal efficiency was calculated to determine the effectiveness of the remediation process according to the following equation:

$$\text{Removal Efficiency (RE \%)} = C0 - RC/\ C0\ X\ 100$$

Where C0 = Initial Concentration before Treatment (Zero Time);
RC= Residual Concentration after Treatment at each Exposure Time.

Temperature, pH & total dissolved solids (TDS)
Temperature, pH and TDS were determined by using digital thermometer and laboratory Bench Meter.

Total suspended solids (TSS)
A known volume of well-mixed sample was filtered through a weighed standard glass-fiber filter (47 mm circles GF/C-Whitman, England) and the solids residue retained on the filter was dried at 105 °C to a constant weight. The increase in weight of the filter represented the total suspended solids according to the following equation.

$$\text{Total Suspended Solids (mg/l)} = \frac{\text{(A-B) X 1000}}{\text{Sample volume (ml)}}$$

Where A = weight of the filter plus the dried residue (mg), and
B = weight of the empty filter (mg).

Fat oil and grease (FOG)
Determination of total content of grease and oily substances were determined using the standard partition gravimetric method described by **Clesceri et al. (1999)**. FOG is calculated according to the following equation:

$$\text{FOG (mg/l)} = \frac{(A - B) \times 1000}{\text{Sample (ml)}}$$

Where A= Weight of the beaker with FOG (mg)
B= Weight of the clean beaker (mg)

Biochemical oxygen demand (BOD_5)
Method 5210 B was used for BOD_5 determination as described in the Standard Methods for Examination of Water and Wastewater (Clesceri *et al*. 1999). BOD_5 can be calculated as follows:

$$BOD_5, \text{ mg/l} = \frac{D1 - D2}{P}$$

Where D1 = DO of diluted sample immediately after preparation in mg/l,
D2 = DO of diluted sample after 5- day incubation at 20 °C in mg/l,
P = Decimal volumetric fraction of sample (300 ml).

Chemical oxygen demand (COD)
Closed Reflux Colorimetric Method 5220 D was used for COD determination using potassium dichromate as chemical oxidant as described in the Standard Methods for Examination of Water and Wastewater **(Clesceri *et al*., 1999)**. Color developed was measured at 620 nm using DR/5000 HACH spectrophotometer DR/2010 HACH spectrophotometer and the concentration was calculated from the slope of the standard curve.

Biological characterization
Total viable count of bacteria (TVC), total coliform (TC) and faecal coliform (FC) were determined in the raw and treated samples as mentioned earlier.

RESULTS
Decontamination of domestic wastewater using batch treatment achieved high removals of all the contaminants but their residuals were still slightly higher than their MPL except for BOD and COD **(El-Bestawy *et al*., 2014)**. Therefore, the mixed culture of the 4 most active selected species fixed as a biofilm and used in continuous mode to enhance the removal efficiency and bring residues of all the contaminants to safe limits. Population dynamics was

determined to define the maturity of the biofilm. Biofilm considered mature after 6 days when bacterial total viable count recorded 3 consecutive readings as shown in **Table 1**. After maturation, raw samples were treated using the biofilm at different flow rates (100, 150 and 200 ml/h) for 6 h with 1 h interval sampling.

Table 1: Population dynamics of the mixed culture during biofilm formation

Time (Day)	Total Viable Count (CFU/ml)
Zero	$6.0x10^8$
1	2.6×10^9
2	3.3×10^9
3	2.2×10^9
4	$7.5\ x10^8$
5	$6.2\ x10^8$
6	$6.0\ x10^8$

pH and DO levels

No significant variations were noticed in the pH values (7.0-7.2) among the tested flow rates, exposure times, proposed system (biofilm or control) before or after the remediation process **(Figure 2A)**. Low DO concentrations (5-9 mg/l) were recorded at the zero time in the raw effluents fed the biofilm system **(Figure 2B)**. DO values were gradually reduced with increasing exposure time in biofilm and control systems reaching their lowest levels (3.5 mg/l) after the 5th h especially in the biofilm system and at the flow rate 200 ml/h. This may be attributed to consumption of DO during biodegradation of the included organic contaminants especially by biofilm bacteria.

Total dissolved solids (TDS)

Using biofilm system enhances the microbial activity towards reduction of TDS **(Figure 3A)**. Raw wastewater effluent had TDS range of 390-420 mg/l at zero time. TDS levels decreased irregularly with time (till the 5^{th} h) at all the tested flow rates and in both biofilm and control systems. Also it is clear that the highest REs were achieved at the slowest flow rate (100 ml/h) while the lowest were achieved at the fastest flow rate (200 ml/h) in the biofilm as well as control systems. RE of TDS using biofilm system ranged between a maximum of 27% (after 5 working hours at 100 ml/h) and a minimum of 7.1% (all the working hours at 200 ml/h). Much lower TDS RE (14.6, 10.3 and 2.4% at 100, 150 and 200 ml/h respectively) were achieved using the control system which confirms the role played by the selected bacteria. TDS levels before and after the treatments were much below their maximum permissible limit (MPL) of 2000 mg/l.

Total suspended solids (TSS)

Raw wastewater recorded TSS level ranged between 140 and 155 mg/l that were gradually decreased with time in both systems (biofilm and control) at all the tested flow rates **(Figure 3B)**. High TSS removals were achieved with significant differences among the different flow rates. Up to 86 % RE of TSS was recorded was by the proposed biofilm at 100 ml/h after 5 h with no significant variations in the TSS removal by control system where 85.2% RE was achieved at 150 ml/h after 5 h. This may be attributed to the relatively low TSS levels in the raw water at the sampling time and the ability of the sand particles of the control system to adsorb such low levels. However, TSS levels in the effluents treated by either the biofilm or control systems were below the MPL (60 mg/l).

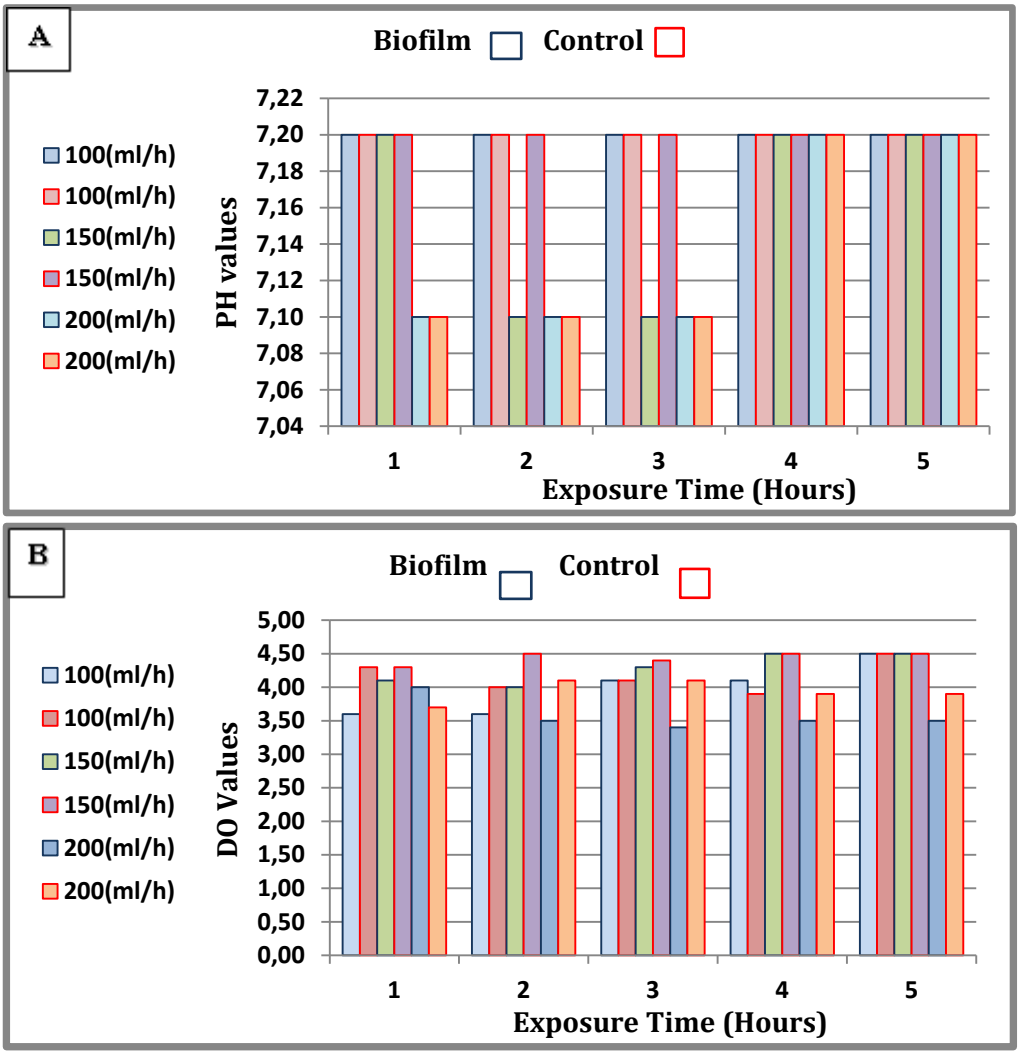

Figure 2. Variation in the (A) pH and (B) DO levels of the raw and treated wastewater using fixed biofilm and control systems at different flow rates and exposure times.

Organic Matter

Biochemical oxygen demand (BOD)

BOD in the raw wastewater recorded a range of 111-120 mg/l that decreased with time in the biofilm and control systems **(Figure 4A)**. Positive relation between RE of the BOD and exposure times was found in both systems (biofilm and control) with no significant variations among the tested flow rates. The flow rate 100 ml/l showed the highest achieved BOD removal (84%) after 5 h in both systems that slightly and insignificantly decreased with increasing the flow rates. It is important here to notice that under all the tested conditions (exposure time, flow rate, seeded and unseeded column) BOD levels decreased much lower than the MPL (60 mg/l) made the effluent very safe to be discharged.

Chemical oxygen demand (COD)

COD in the raw wastewater recorded a range of 350-448 mg/l **(Figure 4B)**. COD removal followed a general increasing trend with increasing exposure time in the biofilm and control systems. In the biofilm system, COD RE recorded 83.7% (73 mg/l) at the lowest flow rate (100 ml/l) after 5 exposure hours. COD residues also showed lower values at 150 and 200 ml/h (70 and 99 mg/l equivalent to 81.6 and 71.7% respectively). These residues are lower

than COD MPL (100 mg/l). Also the control system could reach reasonable RE of the COD reached 79.7% at 150 ml/h after 5 h (77 mg/l) that is compiling with the law.

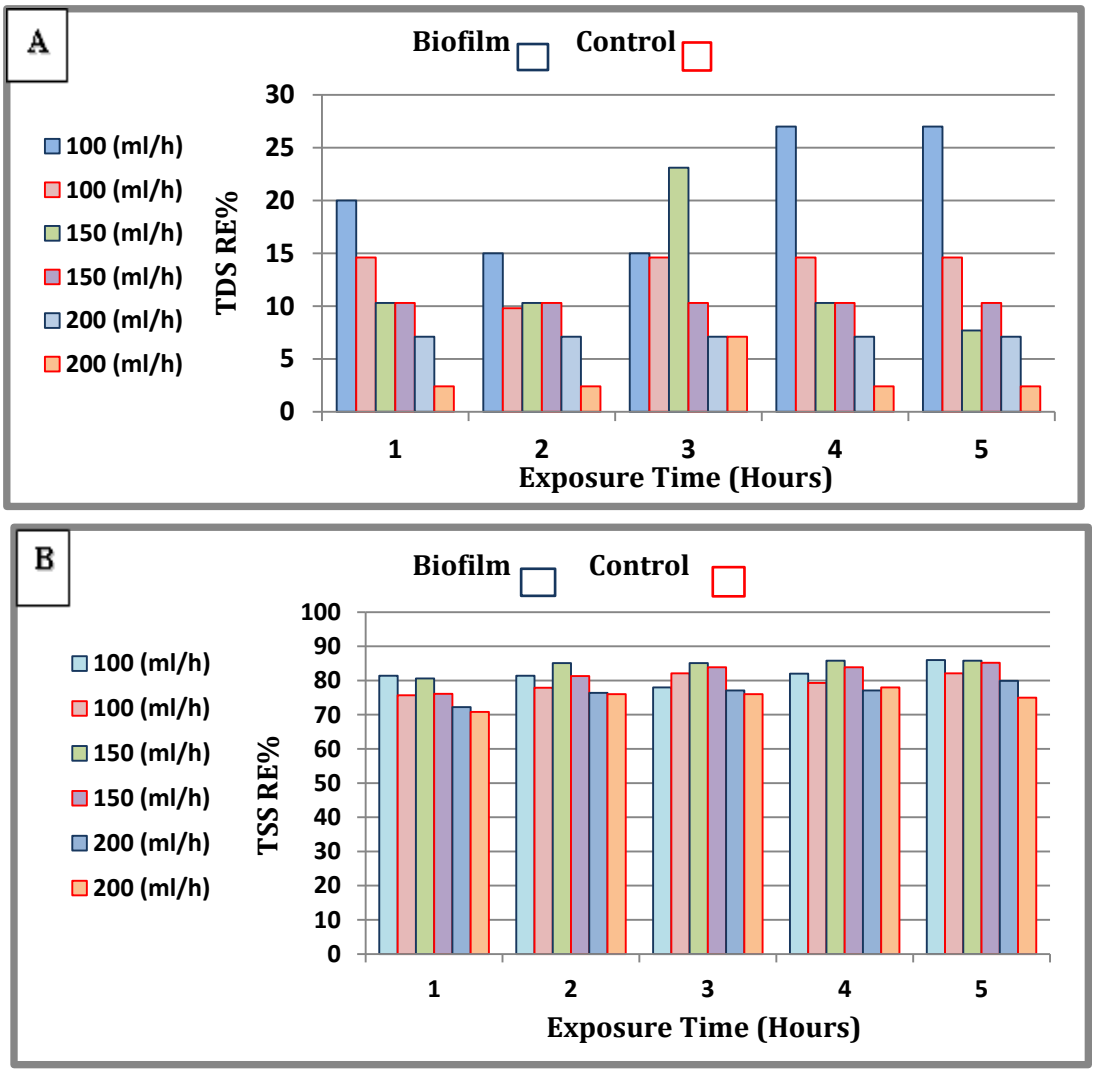

Figure 3. Removal efficiencies (RE %) of wastewater (A) TDS and (B) TSS using biofilm and control systems at different flow rates and exposure times

Fat, Oil and Grease (FOG)

FOG in the raw wastewater recorded a range of 34-38 mg/l **(Figure 4C)**. FOG removal efficiency followed similar trend like the previous parameters where it increased with increasing exposure time in both biofilm and control systems. The highest FOG removal recorded 98.5% (0.4 mg/l) using the biofilm and the control at the flow rate (150 ml/l) after 5 exposure hours. FOG residues at all the tested flow rates by both the biofilm and control from the first running h showed lower values compared to FOG MPL (10 mg/l).

Biological Contaminants

Total Viable Count of Bacteria (TVC)

TVC of the raw and treated wastewater bacteria fluctuated between stimulation and inhibition **(Figure 5A)**. TVC in the wastewater ranged between 4.0 x 10^6 and 16.0 x 10^7 during the experiment. Biofilm system significantly reduced TVC bacteria without any stimulation. On the other hand, bacteria fluctuated between stimulation (75-90% at 200 and 100 m/h respectively after one h) and inhibition in the control system treated samples only especially

at the fastest flow rate. RE (inhibition) of TVC bacteria in the biofilm and control systems regularly increased reached the highest RE (99.8 and 99.7% respectively) after 5 h (last exposure) at the lowest flow rate (100 ml/h) with no variation in TVC RE among the 3 tested flow rates.

Total Coliforms (TC)

Density of total coliform bacteria (TC) ranged in the raw untreated wastewater between 1.0^{8} x 10^{6} and 1.30 x 10^{7} CFU/ml. These values were significantly reduced by the biofilm compared to the control where TC fluctuated between reduction and stimulation especially at 100 m/l flow rate **(Figure 5B)**. The biofilm system achieved 100, 94.4 and 99.2% removals of TC at 100, 150 and 200 ml/h flow rate respectively after 5 h. However, the control system showed high TC stimulation reaching maximum of 13.5 f, 22.2 f and 13.3 f at 100, 150 and 200 ml/h flow rate after 1, 5 and 1 h respectively confirming the suppressive ability of the augmented bacteria in the biofilm to reduce TC bacteria. However, up to 99.9% RE of TC was achieved at 100 ml/h after 5 h followed by 86.3% at 200 ml/h after 2 h and finally 62.9% at 150 ml/h after 1 h.

Fecal Coliforms (FC)

Density of Fecal coliform bacteria (FC) ranged in the raw untreated wastewater between 1.20 **x** 10^{4} and 2.0 **x** 10^{5} CFU/ml **(Figure 5C).** Biofilm system achieved as high as 99.8% RE of the TC at the lowest flow rate (100 ml/h) after 2 h as well as 99.6 and 95% removals at 150 and 200 ml/h after 5 and 1 h respectively. Lower **FC** REs were achieved using the control system with maximum REs of 90.0, 87.4 and 71.0% at 150, 100 and 200 ml/h after 1, 1 and 5 h respectively. According to the MPL of TC, that includes both fecal and non-fecal colifofrm, (5000 CFU/100 ml, i.e. $5x10^{5}$ CFU/ml), TC in the treated sample reached 0.0 CFU/ml and FC reached 1.90 x 10^{2} CFU/ml both of which are much lower than to their MPL. These results confirmed the effectiveness of the proposed biofilm system in the treatment and producing high quality domestic wastewater compared to the batch treatment.

DISCUSSION

Molecular characterization of the 4 most active bacteria identified them as ***Bacillus* sp** (Rz6), ***Bacillus cereus*** (Rz7), ***Bacillus amyloliquefaciens*** (S1) and ***Pseudomonas stutzeri*** (PS). The **mixed culture** (combination with a four selected cultures) proved to be the most efficient for decontamination of domestic wastewater in the present study. ***Bacillus* spp**. such as Rz6 and **S1** are well known as highly resistant spore-forming bacteria that possess excellent characteristics and extremely efficient for many agricultural **(Merritt *et al.*, 1989; Turner** and **Backman, 1991; Powell** and **Jutsum, 1993; Dingman, 1994; Osburn *et al.*, 2011; Emmert** and **Handelsman, 1999; Greene *et al.*, 2001; Nunez-Valdez *et al.*, 2001)**, environmental **(El-Bestawy *et al.*, 2002)** and industrial applications **(Fiechter, 1992; Lee, 1996; Sabir** and **El-Bestawy 2009)**.

Pseudomonas stutzeri (PS) is a Gram-negative, rod-shaped, motile, single polar-flagellated, soil denitrifying bacterium **(Anzai *et al.*, 2000; Lalucat *et al.*, 2006)**. ***Pseudomonas*** species including ***P. stutzeri*** characterized by superior biodegradation and transformation ability for many environmental pollutants **(El-Bestawy** and **Ibrahim, 2005; El-Bestawy *et al.*, 2005**; **El-Bestawy** and **Hans-Jorgen Albrechtsen 2007).**

Environmental laws in **Egypt** and **Saudi Arabia** stated MPLs of the different water contaminants for safe discharge into the open environment. These limits are set to minimize the ecological disturbances and protect aquatic as well as soil environments from hazardous

discharges. The aim of the present work was to design an efficient treatment process for reducing and/or eliminating chemical, biological and organic load from the drainage network in Jeddah City to minimize the environmental impact on the receiving ecosystem.

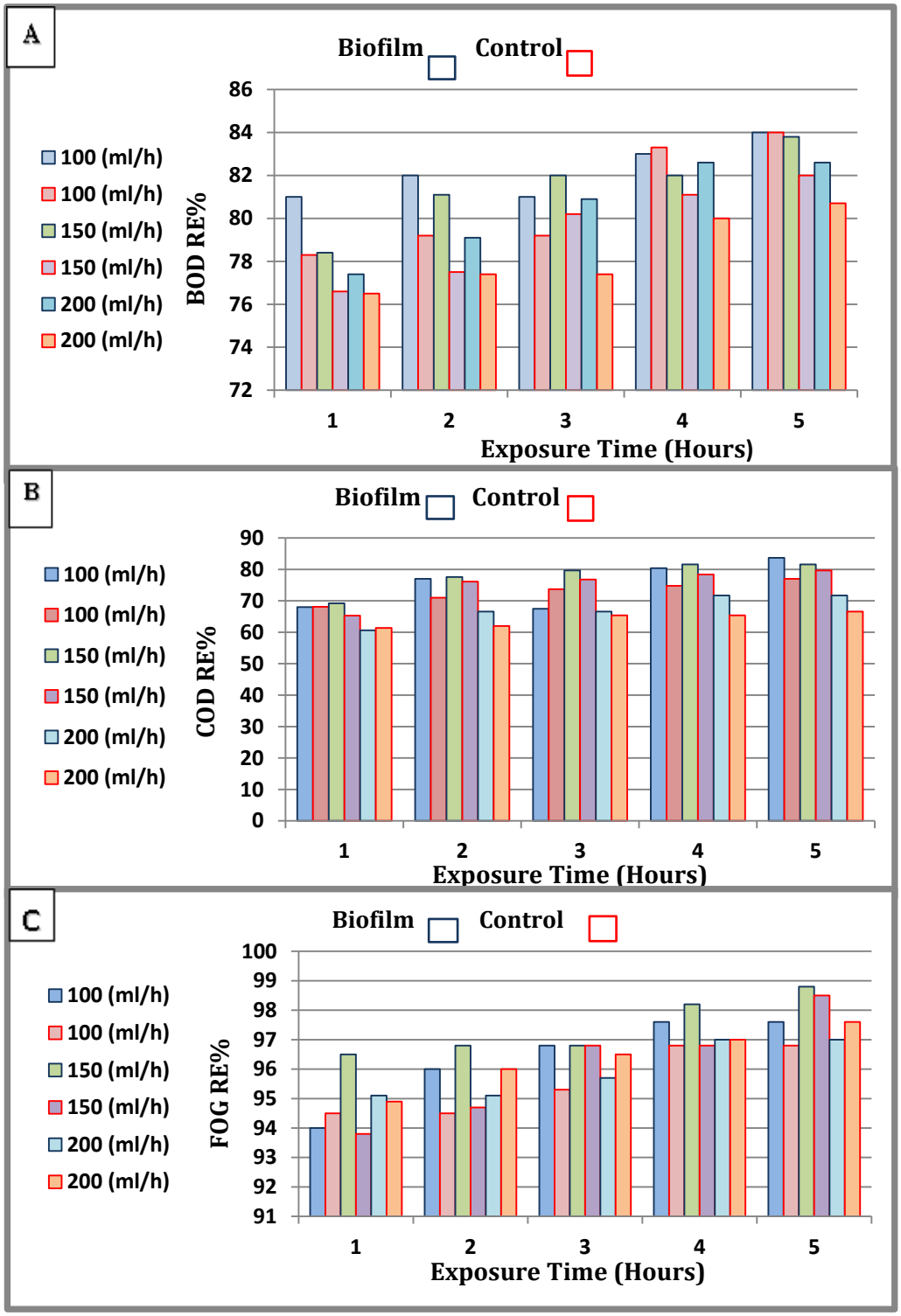

Figure 4. Removal efficiencies (RE %) of wastewater (A) BOD, (B) COD and (C) FOG using biofilm and control systems at different flow rates and exposure times.

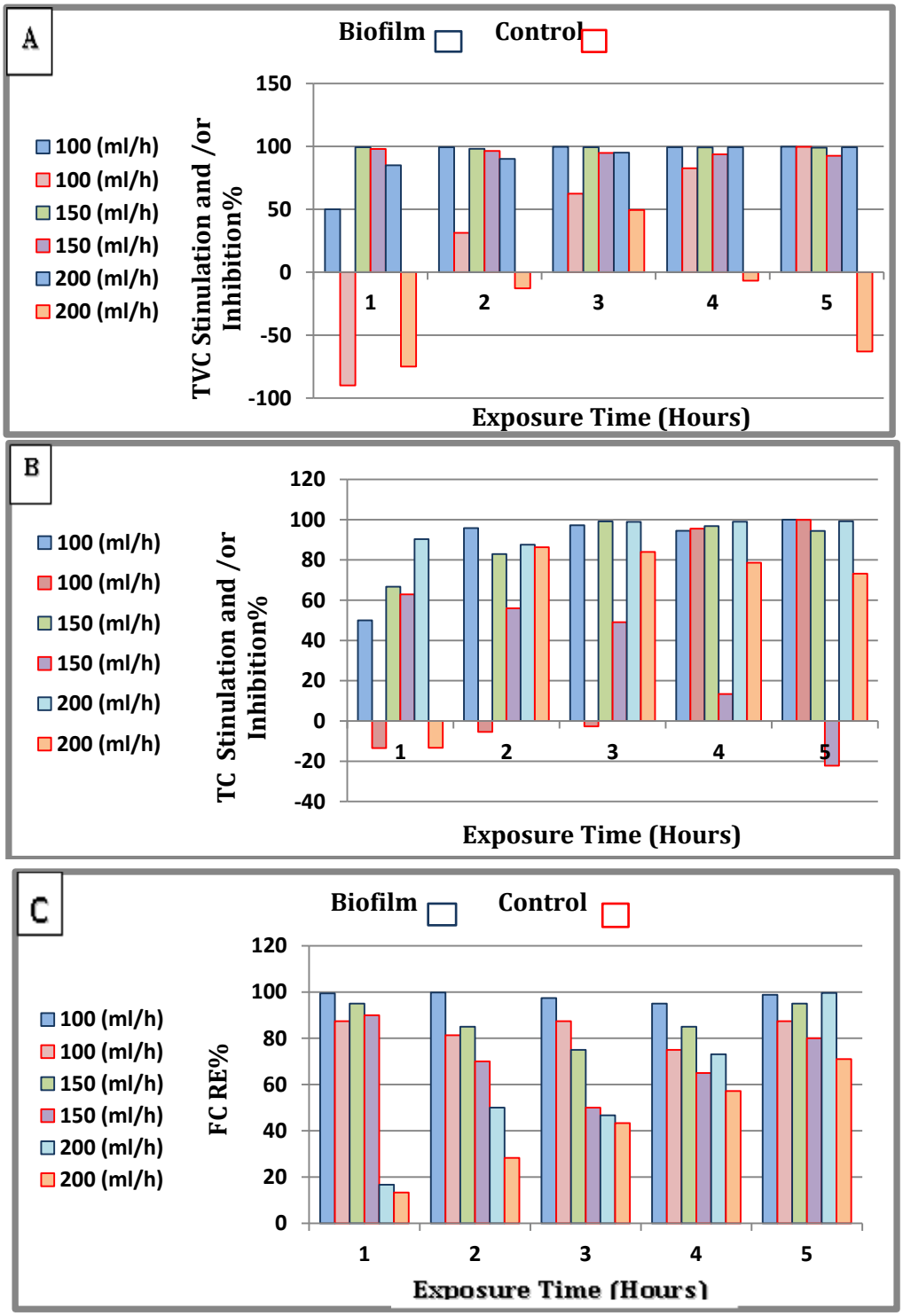

Figure 5. Stimulation/inhibition/removal efficiency of (A) TVC, (B) TC and (C) FC in the raw and treated wastewater at the different flow rates and exposure times.

Biofilm (form of microbial fixation) provides biological treatment with many advantages over their free living counterparts. These advantages include enhancement of contaminants removal **(Nicolella *et al.*, 2000; Gerardi *et al.*, 2006; Hibiya *et al.*, 2004)**, reduction of treatment time, protection of biofilm bacteria from effluent toxicity **(Narmadha *et al.*, 2012)**, death and the wash out of bacterial cells **(Liu *et al.*, 2004)**. In the present study these

advantages were clearly shown where higher RE (s) for all the tested parameters coupled with shortening of the treatment time (5 h) instead of 7 days.

The mixed culture was selected to be fixed in a biofilm system based on its high performance in the removal of all the contaminants during the batch treatment **(El-Bestawy *et al.*, 2014)**. This finding is supported by many workers who documented the superior resistance and ability of microbial consortia in the degradation and accumulation of environmental pollutants **(Gebara, 1999)**. In the present study ***mixed culture*** was able to deal efficiently with polluting contents such as organic matter and pathogenic bacteria. There were two general trends during the treatment with biofilm **(Reddy *et al.*, 2005)**. The first trend was increasing the RE of all the tested parameters with time increase and the second was the huge variations in the RE of all the tested parameters achieved by ***mixed culture*** biofilm system compared to those obtained by the control (bacteria-free system) confirming the efficient role of ***mixed culture*** in removing effluent contaminants **(Lee *et al.*, 1996)**. Also, clear variations were noticed in the RE of all the tested parameters at the different flow rates with no specific trends where the highest REs were achieved at the lower flow rates for some parameters and at the higher flow rates for other parameters **(Kelly *et al.*, 2004)**.

High contaminants concentrations were determined in the raw wastewater. This was shown by the following ranges (mg/l): 144-140 (TSS), 420- 410 (TDS); 115-120 (BOD); 350-448 (COD); 37-38 (FOG); $4.0x10^6$ - $16.0x10^7$ (CFU/ml TVC); $3.0x10^6$ - $1.30x10^7$ (CFU/ml TC) and $1.20x10^4$ - $8.0x10^4$ (CFU/ml FC) in the raw wastewater. The highest achieved RE(s) by the biofilm system after 5 h were 27.0, 86.0, 84.0, 83.7 and 99.8% for TDS, TSS, BOD, COD and TVC respectively at 100 ml/h. FOG recorded 98.8% removal at 150 ml/h. Huge amounts of the tested contaminants were removed in such short time leaving residues (mg/l) of 300 (TDS); 20 (TSS); 19 (BOD); 73 (COD); 0.4 (FOG); $2.6x10^5$ CFU/ml (TVC); zero CFU/ml (TC) and $5.0x10^1$ CFU/ml (FC) by the mixed culture. These results are supported by other workers **(Hsien** and **Lin, 2005)**.

On the other hand, the highest achieved RE(s) by the control system after 5 h were 14.6, 85.2, 84.0, 79.7, 99.7, 99.9 and 87.4 % for TDS, TSS, BOD, COD, TVC, TC and FC respectively at 100 CFU/h while 98.5 RE% was achieved for FOG at 200 ml/h. The amounts of the tested contaminants were removed by the control system leaving residues (mg/l) of 350 (TDS), 23 (TSS); 19 (BOD); 77 (COD); 0.5 (FOG); 5.1×10^5 CFU/ml (TVC); 4.00×10^3 CFU/ml (TC) and 1.01×10^4 CFU/ml (FC) of wastewater bacteria. Results proved that the proposed biofilm system is very efficient for treating the wastewater effluents and confirmed the ability of the selected bacteria for the removal of the target contaminants especially pathogenic bacteria (coliform). This system could reach higher removal for all the tested parameters reaching acceptable limits for safe discharge.

CONCLUSION

Wastewater in the present study showed high levels of all the tested parameters that poses high pollution potential and dangerous effects on the receiving environments and also creates many difficulties in the treatment facilities. Fixation of bacteria on solid medium as a biofilm showed many advantages over their planktonic free living counterparts. It enhances the bacterial growth, reduces wastewater toxicity and increase bacterial resistance towards the involved contaminants. Considering the very short time that biofilm runs for (5 h), it seems that the proposed biofilm system is very efficient for treating the wastewater effluents.

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