



## 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:  $4.00 \times 10^6$  and  $16.0 \times 10^7$ ), 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 REs by the proposed biofilm system were 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 hours at the lowest tested flow rate (100 mL/h) except for FOG (150 mL/L). Treatment using the biofilm system has decreased all the tested pollutants to much lower levels than the maximum permissible limits 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.

**Keywords:** Biofilm; Bacteria; Consortium; Contamination; Domestic wastewater; Treatment

### 1. 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 blackwater, is correctly the subset of

wastewater that is contaminated with feces or urine, but is often used to mean any wastewater [1,2]. Human waste 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 [3].

Sewage composition varies widely but may contain more than 95% water with pathogens (bacteria, viruses, parasitic

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worms, protozoa and helminths) [4–8] and non-pathogenic bacteria (>100,000 CFU/mL for sewage). Chemically, sewage water is a complex matrix, involving 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 fibers, plant's materials, 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.), macrosolids (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.) [5,9–11].

Sewage containing 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 [12], organic matter and biological contaminants [6,13–17] were discussed earlier.

Sewage or domestic wastewater treatment is considered as 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 reuse or discharge back into the environment. Although conventional sewage treatment involves primary, secondary and tertiary treatment stages, secondary (biological) treatment is considered as the main process where it removes dissolved and suspended biological matter and is typically performed by indigenous, waterborne microorganisms in a managed habitat [18,19]. 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 [20,21].

Secondary treatment systems may be designed as fixed-film or suspended growth secondary treatment systems (activated sludge and surface aerated basins) [22,23]. In contrary to their planktonic (free-living) counterparts, bacteria within biofilms are remarkably resistant to many natural and artificial factors including traditional antimicrobial agents [24,25] and disinfectants [26], since they are protected by extracellular polymers. In the industrial and medical sectors, microbial biofilms represent a major challenge. Therefore, serious attempts to monitor [27,28], control and/or prevent their development were investigated using different agents such as the fungicides pyrimethanil and carvacrol [29,30] or using very promising biofilm dispersal agents such as nitric oxide (NO) encapsulated within a hydrogel composed of cellulose nanocrystals [26]. Moreover, different enzymes such as pectin methyltransferase [31], 5 kDa peptide fraction of the cytosol from sea-cucumber *Holothuria tubulosa* coelomocytes [32] and Paracentrin 1 from the 5 kDa peptide fraction from the coelomocyte cytosol of the sea-urchin *Paracentrotus lividus* [33]

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 and remove hazardous toxic contaminants such as heavy metals even at high concentrations [34]. Biofilms are also the most reactive component in natural aquatic environments where they perform indispensable roles in cycling of essential elements such as carbon and nitrogen as well as biodegradation of pollutant organic wastes [35]. Microbial N-cycling *nirS* and *nirK* (denitrification through the conversion of  $\text{NO}_2$  to  $\text{NO}$ ), *nifH* ( $\text{N}_2$  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 [36]. Moreover, 13 marine bacterial biofilm strains have been isolated from different inert surfaces immersed in the Mediterranean Sea at the Toulon Bay (France). They were belonged 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 [37]. Genetic engineering of biofilm strains can remarkably enhance their resistance and ability to degrade environmental contaminants [38,39].

Bioremediation is considered as a novel, efficient and environmentally safe technology for natural inexpensive decontamination of polluted systems. It is used in the Middle East in enhancement of food, feed, fertilizer production and pollution control.

Application of bioremediation using indigenous microorganisms for decontamination of water systems polluted with organic contaminants provides a viable, efficient pollution control and sustainable approach for environmental resources.

Therefore, the present study aimed to search, develop and investigate novel treatment technology with improved antimicrobial activity against pathogens as alternatives to conventional methods for decontamination of domestic wastewater. The proposed treatment system manipulate the ability of multispecies bacterial biofilm involved *Pseudomonas stutzeri* M15-10-3 (PS), *Bacillus* sp. OU-40 (Rz6), *Bacillus amyloliquefaciens* T004 (S1) and *Bacillus cereus* OPP5 (Rz7). Such system gathered the marvellous advantages of the efficient bacterial biofilm and the well-known capabilities of *Pseudomonas* and *Bacillus* species as antimicrobial and biodegrading agents.

## 2. Materials and methods

### 2.1. Sampling

Raw 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 presterilized 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 select the best treatment technology. In addition, post-treatment characterization took place in order to evaluate treatment efficiency.

## 2.2. 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 [40] 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 mixed culture 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 basis. S1 and PS were originally isolated from heavily polluted media (wastewater and environments) and previously exhibited superior pollution decontaminating ability [40,41].

## 2.3. Media preparation and culturing conditions

Nutrient broth and nutrient agar (NB and 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 121°C for 20 min and used freshly for growth experiments as well as biodegradation assays.

Total coliform (TC) and fecal coliform (FC) bacteria were determined using Chapman TTC Agar (Tergitol® 7 Agar), 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 h.

## 2.4. Bacterial counting

Changes in the total heterotrophic bacterial viable counts during wastewater treatment were determined using pour plate technique of the standard plate count method after sequential dilutions [42]. Samples were cultured in NA medium and incubated at 37°C for 24 h. Colony forming units (CFU) of the bacterial 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 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. TC 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 h incubation at 37°C. The same technique was performed for determination of FC bacteria after incubation for 24 h at 45°C. FC bacteria took various shades of blue. Non-FC colonies were gray to cream. All samples were analyzed in triplicate.

## 2.5. Bioremediation bioassay using free-living bacteria (batch mode)

This experiment was previously performed by El Bestawy et al. [40]. Indigenous as well as exogenous bacteria were tested 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. Samples were collected daily for a week, characterized and removal efficiencies (REs) were calculated after which four promising isolates for bioremediation process were selected.

## 2.6. Biofilm system development

Based on the results of the free-living bioassay [40] and the RE 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 with the individual strains. Prior to remediation experiment, a liquid mixed culture was prepared in NB medium and incubated overnight.

Two cylindrical glass columns (30 × 7 cm) were sealed at the bottom by a porous net ( $d < 1$  mm) 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 for three times at 121°C



Fig. 1. The biofilm system.

for 1 h. 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 ( $6.0 \times 10^8$  CFU/mL) and left 10 d to allow bacterial cells adhesion forming the biofilm. The two columns were connected with an upflow air supply, which was adjusted to operate alternately for 1 h and pause for 2 h.

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

### 2.7. Bioremediation bioassay using fixed bacteria (biofilm)

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 the both biofilm and bacteria-free (control) columns at 30 min interval for 2.5 h. 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.

### 2.8. 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 (DO) content, total suspended solids (TSS), total dissolved solids (TDS), biochemical oxygen demand ( $BOD_5$ ), chemical oxygen demand (COD), fat, oil and grease (FOG), total viable count (TVC) of bacteria, count of TC and FC bacteria all of which were determined using the standard techniques described in Standard Methods for the Examination of Water and Wastewater [42]. After treatment the selected parameters were analyzed to determine their residual levels at each exposure time. RE was calculated to determine the effectiveness of the remediation process according to the following equation:

$$\text{Removal efficiency (RE \%)} = C_0 - RC/C_0 \times 100 \quad (1)$$

where  $C_0$  is the initial concentration before treatment (zero time) and RC is the residual concentration after treatment at each exposure time.

### 2.9. Temperature, pH and total dissolved solids

Temperature, pH and TDS were determined by using digital thermometer and laboratory Bench Meter.

### 2.10. Total suspended solids

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 solid residue retained on the filter was dried at 105°C to a constant weight. The

increase in weight of the filter represented the TSS according to the following equation:

$$\text{Total suspended solids (mg/L)} = \frac{(A - B) \times 1,000}{\text{Sample volume (mL)}} \quad (2)$$

where  $A$  is the weight of the filter plus the dried residue (mg) and  $B$  is the weight of the empty filter (mg).

### 2.11. Fat, oil and grease

Determination of total content of grease and oily substances were determined using the standard partition gravimetric method described by Clesceri et al. [42]. FOG is calculated according to the following equation:

$$\text{FOG (mg/L)} = \frac{(A - B) \times 1,000}{\text{Sample (mL)}} \quad (3)$$

where  $A$  is the weight of the beaker with FOG (mg) and  $B$  is the weight of the clean beaker (mg)

### 2.12. Biochemical oxygen demand

Method 5210 B was used for  $BOD_5$  determination as described in the Standard Methods for Examination of Water and Wastewater [42].  $BOD_5$  can be calculated as follows:

$$\text{BOD}_5, \text{mg/l} = \frac{D_1 - D_2}{P} \quad (4)$$

where  $D_1$  is the DO of diluted sample immediately after preparation in mg/L,  $D_2$  is the DO of diluted sample after 5 d incubation at 20°C in mg/L and  $P$  is the decimal volumetric fraction of sample (300 mL).

### 2.13. Chemical oxygen demand

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 [42]. Color developed was measured at 620 nm using DR/5000 HACH spectrophotometer and the concentration was calculated from the slope of the standard curve.

### 2.14. Biological characterization

TVC of bacteria, TC and FC were determined in the raw and treated samples as mentioned earlier.

## 3. Results

Decontamination of domestic wastewater using batch treatment achieved high removals of all the contaminants but their residuals were still slightly higher than their maximum permissible limit (MPL) except for BOD and COD [40]. Therefore, the mixed culture of the four most active selected species fixed as a biofilm and used in continuous mode to

enhance the RE 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 d when bacterial TVC recorded three 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.

### 3.1. pH and DO levels

Almost no 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 (Fig. 2(A)). Low DO concentrations (5.9 mg/L) were recorded at the zero time in the raw effluents fed the biofilm system (Fig. 2(B)). DO values were gradually reduced with increasing exposure time in biofilm and control systems reaching their lowest levels (3.5 mg/L) after the fifth hour especially in the

Table 1  
Population dynamics of the mixed culture during biofilm formation

Time (d)	Total viable count (CFU/mL)
Zero	$6.0 \times 10^8 \pm 0.2 \times 10^8$
1	$2.6 \times 10^9 \pm 0.1 \times 10^9$
2	$3.3 \times 10^9 \pm 0.13 \times 10^9$
3	$2.2 \times 10^9 \pm 0.13 \times 10^9$
4	$7.5 \times 10^8 \pm 0.05 \times 10^8$
5	$6.2 \times 10^8 \pm 0.076 \times 10^8$
6	$6.0 \times 10^8 \pm 0.1 \times 10^8$

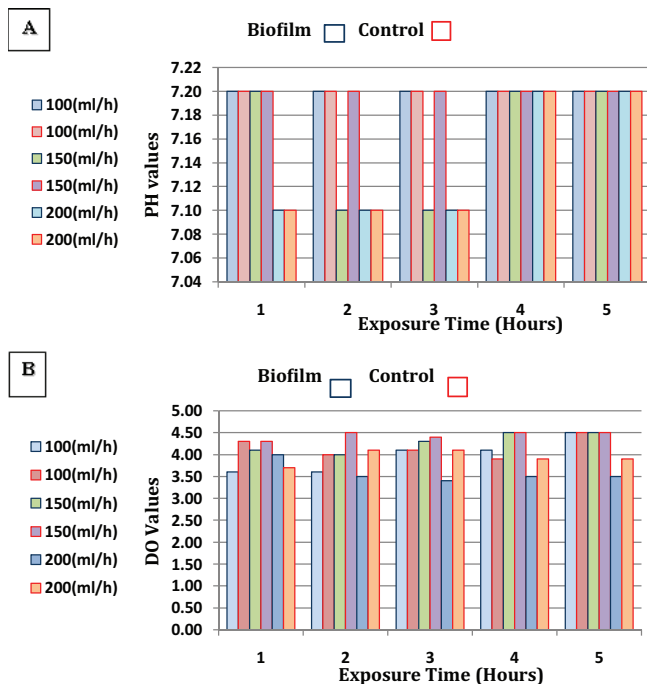


Fig. 2. Variation in the (A) pH and (B) DO levels of the raw and treated wastewater using fixed biofilm and control systems at different flow rates and exposure times.

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.

### 3.2. Total dissolved solids

Using biofilm system enhances the microbial activity toward reduction of TDS (Fig. 3(A)). Raw wastewater effluent had TDS in the range of 390–420 mg/L at zero time. TDS levels decreased irregularly with time (till the fifth hour) at all the tested flow rates and in both biofilm and control systems. In addition, it is clear that the highest REs were achieved at the slowest flow rate (100 mL/h) while the lowest REs 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 MPL of 2,000 mg/L.

### 3.3. Total suspended solids

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 (Fig. 3(B)). High TSS removals were achieved with significant differences among the different flow rates. Up to 86% RE of TSS was recorded by the proposed biofilm at 100 mL/h after 5 h with no big 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

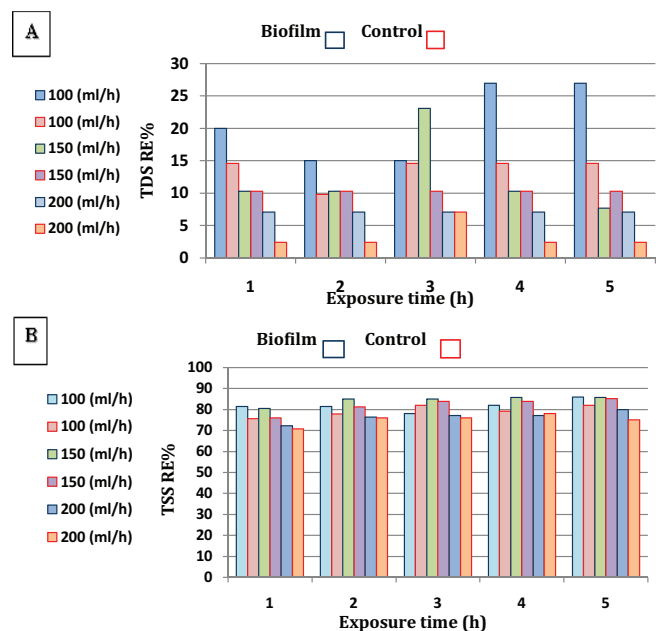


Fig. 3. Removal efficiencies (REs %) of wastewater (A) TDS and (B) TSS using biofilm and control systems at different flow rates and exposure times.

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).

### 3.4. Organic matter

#### 3.4.1. Biochemical oxygen demand

BOD in the raw wastewater recorded in the range of 111–120 mg/L that decreased with time in the biofilm and control systems (Fig. 4(A)). RE of the BOD proportionally increased with exposure times 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.

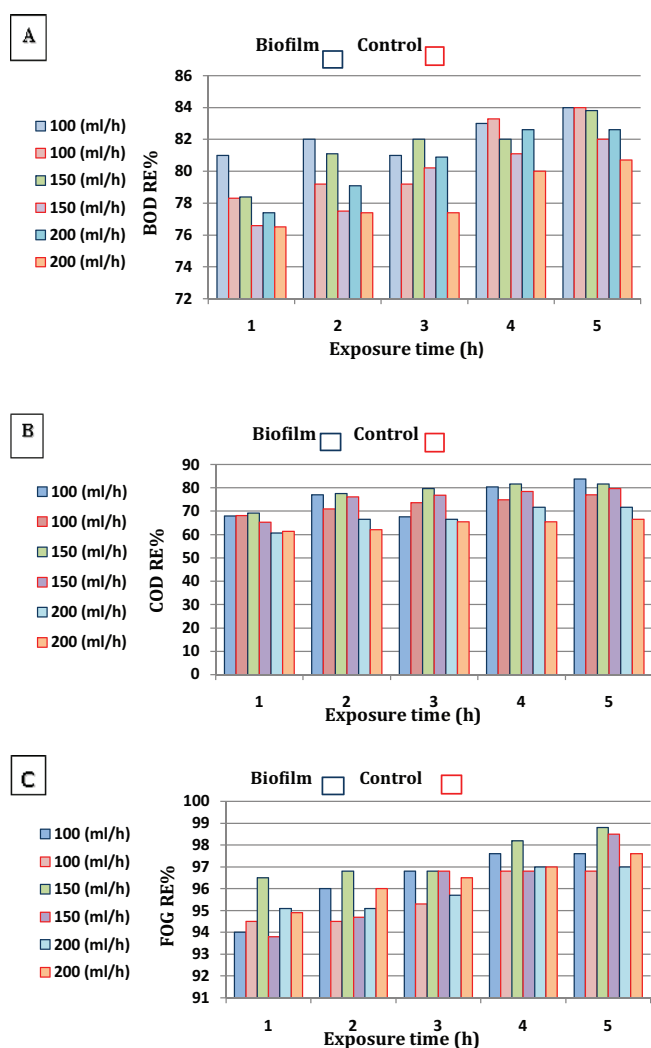


Fig. 4. Removal efficiencies (REs %) of wastewater (A) BOD, (B) COD and (C) FOG using biofilm and control systems at different flow rates and exposure times.

#### 3.4.2. Chemical oxygen demand

COD in the raw wastewater recorded in the range of 350–448 mg/L (Fig. 4(B)). 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 h of exposure. 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). In addition, the control system could reach reasonable RE of the COD reached 79.7% at 150 mL/h after 5 h (77 mg/L) that compile with the law.

#### 3.4.3. Fat, oil and grease

FOG in the raw wastewater recorded in the range of 34–38 mg/L (Fig. 4(C)). FOG RE followed similar trend to 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 h of exposure. FOG residues at all the tested flow rates by the both biofilm and control from the first running hour showed lower values compared with FOG MPL (10 mg/L).

### 3.5. Biological contaminants

#### 3.5.1. Total viable count of bacteria

TVC of the raw and treated wastewater bacteria fluctuated between stimulation and inhibition (Fig. 5(A)). TVC in the wastewater ranged between  $4.0 \times 10^6$  and  $16.0 \times 10^7$  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 mL/h, respectively, after 1 h) and inhibition only in the control system treated samples 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 three tested flow rates.

#### 3.5.2. Total coliforms

Density of TC bacteria ranged in the raw untreated wastewater between  $1.08 \times 10^6$  and  $1.30 \times 10^7$  CFU/mL. These values were significantly reduced by the biofilm compared with the control where TC fluctuated between reduction and stimulation especially at 100 mL/L flow rate (Fig. 5(B)). 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-, 22.2- and 13.3-fold 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.

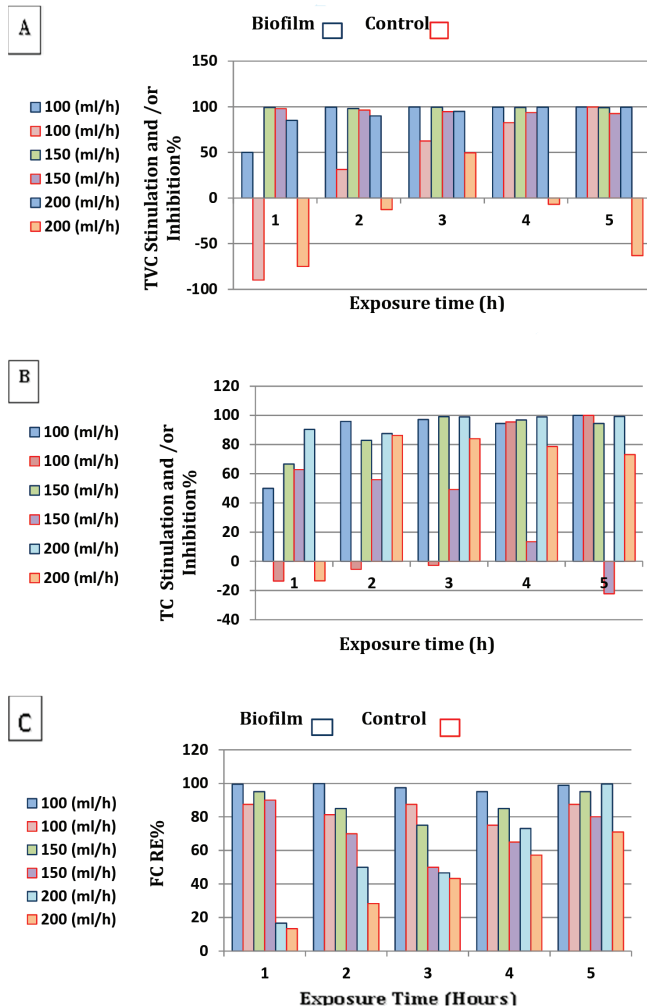


Fig. 5. Stimulation/inhibition/removal efficiency of (A) TVC, (B) TC and (C) FC in the raw and treated wastewater at the different flow rates and exposure times.

### 3.5.3. Fecal coliforms

Density of FC bacteria ranged in the raw untreated wastewater between  $1.20 \times 10^4$  and  $2.0 \times 10^5$  CFU/mL (Fig. 5(C)). 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-FC (5,000 CFU/100 mL, i.e.,  $5 \times 10^5$  CFU/mL), TC in the treated sample reached 0.0 CFU/mL and FC reached  $1.90 \times 10^2$  CFU/mL both of which are much lower than their MPL. These results confirmed the effectiveness of the proposed biofilm system in the treatment and producing high quality domestic wastewater compared with the batch treatment.

## 4. Discussion

Molecular characterization of the four most active bacteria identified them as *Bacillus* sp. (Rz6), *B. cereus* (Rz7),

*B. amyloliquefaciens* (S1) and *P. 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 [43–50], environmental [51] and industrial applications [52–54].

*P. stutzeri* (PS) is a Gram-negative, rod-shaped, motile, single polar-flagellated and soil denitrifying bacterium [55,56]. *Pseudomonas* species including *P. stutzeri* characterized by superior biodegradation and transformation ability for many environmental pollutants [57–59].

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. Biofilm (form of microbial fixation) provides biological treatment with many advantages over their free-living counterparts. These advantages include enhancement of contaminants removal [60–62], reduction of treatment time, protection of biofilm bacteria from effluent toxicity [63], death and the wash out of bacterial cells [64]. 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 d were achieved using the biofilm system.

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 [40]. 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 [65]. In the present study, the *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 [66]. 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 with those obtained by the control (bacteria-free system) confirming the efficient role of the *mixed culture* in removing effluent contaminants [67]. In addition, 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 [68].

Contaminants concentrations determined in the raw wastewater were in the following ranges (mg/L): 144–140 (TSS), 420–410 (TDS), 115–120 (BOD), 350–448 (COD), 37–38 (FOG), TVC:  $4.0 \times 10^6$  to  $16.0 \times 10^7$  (CFU/mL), TC:  $3.0 \times 10^6$  to  $1.30 \times 10^7$  (CFU/mL) and FC:  $1.20 \times 10^4$  to  $8.0 \times 10^4$  (CFU/mL). 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. Such achievements at the lowest flow rate is mainly attributed to longer exposure time with biofilm bacteria compared with

the other tested flow rates (150 and 200 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),  $2.6 \times 10^5$  CFU/mL (TVC), 0 CFU/mL (TC) and  $5.0 \times 10^1$  CFU/mL (FC) by the mixed culture. These results are supported by other workers [69]. In contrast,  $88.6\% \pm 3.7\%$ ,  $86.3\% \pm 4.9\%$ ,  $91.2\% \pm 9.7\%$  as well as  $>90\%$  RE were obtained for COD, BOD, TSS and indicator organism and pathogens using a uniquely designed two-stage system (a modified septic tank followed by an upflow anaerobic filter) [70]. Such results confirmed the superior results of the proposed biofilm systems which achieved almost similar and even higher pathogens removal (99.8%) after only 5 h instead of 120 d. Much lower removals were recorded by conventional and baffled septic tanks. RE of 53.4%, 56% and 65.3% was recorded for COD at hydraulic retention time (HRT) of 24, 48 and 72 h, respectively, with residual COD of 412, 380 and 334 mg/L, respectively. While 68.4%, 57, 53.5% were achieved for BOD and 65.3%, 58.3%, 55% for TSS at HRTs of 72, 48 and 24 h, respectively [71]. Temperature, initial influent COD and TSS may also affect the treatment performance. In a recent study using laboratory septic tank, RE ranges of 74%–86% and 86%–88% were obtained for COD and TSS, respectively, at temperature in the range of 15°C–5°C and long HRT of 36 or 50 d. Increasing COD of influent from 450 to 4,000 mg/L resulted in a decreased performance of the septic tank (to ~25% less) [72]. Comparing such results with those obtained in the present study at the very short time proved the proposed treatment system is highly advantageous over other systems.

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 mL/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),  $5.1 \times 10^5$  CFU/mL (TVC),  $4.00 \times 10^3$  CFU/mL (TC) and  $1.01 \times 10^4$  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.

## 5. 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 toward 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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