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# Decontamination of Domestic Wastewater Using Suspended Individual and Mixed Bacteria in Batch System

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## Abstract

The study aimed to investigate the ability of indigenous and/or exogenous free living bacteria either individual or as mixed culture to decontaminate raw domestic wastewater. Seven indigenous and two exogenous bacteria were selected and identified using traditional as well as molecular characterization, then used in the batch remediation system for seven days. Results indicated that the raw wastewater was relatively of high strength according to the levels of all the tested parameters. Treatment efficiency was time and bacterial species dependent. In general, the mixture of the tested bacteria considered the most efficient for the removal of all the tested parameters. *Pseudomonas stutzeri* (PS) was perfect for removing organic matter (BOD and COD) while the mixed culture considered the most efficient for removing fecal coliform (≈100%) brought them to safe (60, 100 mg/l and ≈ 0.0 CFU/ml respectively) discharge limits (MPL) stated by the Egyptian and Saudi Environmental laws that regulate discharging of domestic and industrial wastewater into fresh and saline open water. In addition, high removal efficiencies of TSS, FOG and TC recording 39.1, 90.0 and 99.0% respectively were achieved by *B. amyloliquefaciens* (S1), E. coli (Rz6) and the mixed culture respectively. However, their residuals still higher (23.3, 20 and 200 fold respectively) than their MPLs for the safe discharge due to the short treatment course. Therefore, longer treatment time and/or using biofilm of the selected bacteria are highly recommended to bring the contaminated domestic effluent it to the safe limits for the environment. The present study confirmed the ability of the selected bacteria for the removal of the target contaminants especially pathogenic bacteria (coliform) and thus can be manipulated efficiently to decontaminate polluted systems providing the optimum degradation conditions.

**Keywords:** Bacteria; Batch process; Biological treatment; BOD; COD; Domestic wastewater; Individual; Mixed cultures

## Introduction

Domestic or sewage wastewater is any water that has been adversely contaminated with faeces or urine. Sewage treatment is the process of removing contaminants from wastewater, including household sewage and runoff effluents aiming to produce an environmentally safe treated effluent or sludge suitable for disposal or reuse (usually as farm fertilizer). With proper technology, it is possible to re-use sewage effluent for drinking water especially and usually only in places with limited water supplies [1]. Sewage is generated by residential, institutional, commercial and industrial establishments and includes household liquid wastes from toilets, baths, showers, kitchens and sinks that is disposed of via sewers. Its composition varies widely but may contain more than 95% water with pathogens (bacteria, viruses and parasitic worms) 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 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 toxins (pesticides, poisons, herbicides, etc.) [2,3].

Domestic sewage can be treated physically, chemically or biologically to remove the included contaminants [4]. It can be treated close to where it is created (in septic tanks, biofilters or aerobic treatment systems), or collected and transported via a network of pipes and pump stations to a municipal treatment plant. Industrial sources of wastewater often require specialized treatment processes [5,6]. 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 [4,7]. 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. Secondary treatment systems may be designed as fixed-film or suspended growth secondary treatment systems (activated sludge and surface aerated basins) [8,9].

Bioremediation is a superior destruction technology for some types of hazardous wastes (particularly those contaminated with organic

chemicals). It is also seen as environmentally friendly, cost effective and low energy/chemicals consuming technology. The main objective of present study was to investigate the ability of some free-living indigenous and exogenous bacteria (pure and/or mixed cultures) for remediation and disinfection of the raw sanitary effluent of Jeddah City before discharging into the open environments.

## Materials and Methods

### Sampling

Sewage samples were collected from the drainage network in Jeddah City, Saudi Arabia in pre-sterilized bottles. Temperature and pH were measured in the field at the collection points.

### Microorganisms

Nine bacterial species, seven indigenous (Rz1-Rz7) isolated from the collected sewage samples and two exogenous (S1 and PS) provided by the Institute of Graduate Studies and Research, Alexandria University (IGSR) were used in the present study. S1 and PS were previously identified as *Bacillus* sp. and *Pseudomonas* sp. respectively. They were isolated from heavily polluted wastewater and environments [9-14]. Cultures were maintained at 4°C on nutrient agar slants and transferred monthly.

### Culture media and growth conditions

Dehydrated nutrient broth (NB) and nutrient agar (NA) (Oxide®) were used as a general medium for enumeration, purification, transferring and preservation of viable bacteria from sewage samples. Total coliform (TC) and fecal coliform (FC) bacteria were determined using coliform selective dehydrated medium (Chapman TTC Agar-Lactose TTC Sodium Heptadecylsulfate Agar) supplied by Scharlau (Spain). After preparation, all media were sterilized by autoclaving at 121°C for 20 min and used freshly. Cultured bacteria were incubated at 37°C for 24 hours. The experimental culture for biodegradation was inoculated with 1% (v/v) inoculum and incubated as indicated in each experiment.

### Determination of coliform bacteria

All the microbiological tests were performed according to the standard techniques described in Standard Methods for the Examination of Water and Wastewater [15]. Determination of the coliform group was performed using membrane filter technique (MF) of the standard coliform count test. A specific volume of the sewage sample was filtered through polycarbonate membrane filter (22 µm) which, incubated on Chapman TTC Agar medium containing lactose for 24 h at 37°C. Coliform bacteria that retained on the filter and grew as red colonies with a metallic (golden) sheen were counted considering dilution factor. The same technique was used for determination of fecal coliform bacteria after incubation for 24 h at 45°C. Fecal coliform bacteria took various shades of blue. Non fecal coliform colonies were grey to cream. All samples were analyzed in triplicates.

### Bacterial identification

Heterotrophic bacterial colonies were purified by streaking on NA agar plates, incubated at 37°C. Purified indigenous and exogenous isolates were identified using cell and colony morphology, differential Gram staining, biochemical profiling using the dehydrated media in the API kits and molecular characterization.

### Molecular identification

Molecular characterization for the most promising indigenous isolates and the exogenous isolates was performed by extraction and purification of the total genomic DNA from 5 ml overnight NB culture using the chromosomal DNA extraction kit (Genomic DNA Purification Kit, Thermo Scientific). PCR was performed in a light cycler Eppendorf PCR machine. A 1300 bp fragment was obtained by PCR amplification of the 16S rDNA gene using the primers F-start: 5'-AGAGTTTGATCMTGGCTCAG-3' and R-1387: 5'-CGGGC GGTGTGTACAAGG-3' [16]. PCR amplification conditions were performed by an initial denaturation step at 94°C for 10 min followed by 30 denaturation cycles at 94°C for 1 min, annealing at 60°C for 1 min and an extension at 72°C for 1 min followed by a final extension step at 72°C for 10 min. Amplicons of 16S rDNA were purified using PCR purification kit (GenJET PCR purification kit, Thermo Scientific). Each of these purified products was sequenced by the chain terminator method (API model 3730 xl, Bioneer, Germany). The resulted DNA sequences were phylogenetically analyzed using the BLAST search program [17]. Multiple sequence alignment and molecular phylogeny were performed using MEGA 5.o software [18].

### Biological treatment of sewage effluents

Four most promising isolates (Rz6, Rz7, S1 and PS) were primarily selected according to visual screening test where capable species could reduce turbidity (increases clarity) of sewage effluents. They were then employed as free-living individuals or mixture for the treatment of the contaminated domestic effluent. Five cultures (4 individual and one mixed) were individually activated in 100 ml (10%) NB medium (3 replica each) and incubated till heavy growth was obtained. Total viable count (TVC) of all cultures was estimated to determine the initial densities of the different inoculum. Bacterial inoculum were individually seeded into 900 ml (90%) raw domestic effluent, previously characterized (zero time or raw readings), reaching a final volume of 1 L. Effluent cultures, individual and mixed as well as a control sample (one litre un-inoculated domestic effluent) were incubated for 7 days under the previously mentioned conditions where samples were aseptically drawn at 24 h interval. Treated effluent' samples were re-characterized where residual levels of the selected parameters were determined at each exposure time and their removal efficiencies were calculated to determine the effectiveness of the remediation process.

### Characterization of wastewater samples

Wastewater was characterized before and after the proposed treatment. Characterization of the wastewater included its pH, temperature, dissolved oxygen content (DO), total dissolved solids (TDS), total suspended solids (TSS), biochemical oxygen demand (BOD), chemical oxygen demand (COD), fat, oil and grease (FOG), all of which were determined using the standard techniques described in Standard Methods for the Examination of Water and Wastewater [15]. Post- treatment characterization determined residual levels of the selected parameters at each exposure time. Removal efficiencies of all parameters were calculated to determine the effectiveness of the remediation process according to the following equation:

Removal Efficiency(RE%)=$C_0$-RC/$C_0$X100

Where $C_0$=Initial Concentration before Treatment (Zero Time);

RC=Residual Concentration after Treatment at each Exposure Time.

## Results

### Isolation and characterization of indigenous bacteria

Colony morphology and the microscopic cell examination as well as Gram stain reaction of the purified bacteria (data not shown) revealed that two of the isolated indigenous bacteria were Gram positive rods (Rz2 and Rz5) and the rest were Gram negative rods. According to the API biochemical profiles (Table 1), indigenous bacteria were most probably affiliated to five species including *Pseudomonas cepacia* (Rz2, Rz6 and Rz7); *Enterobacter agglomerans* (Rz1); *Klebsiella pneumonia* (Rz4*); Salmonella arizonae* (Rz3) and *E. coli vulneris* (Rz5).

| Bacterial Code | API Identification |
|---|---|
| Rz1 | *Enterobacter agglomerans* Id=43.1% |
| | *E. cloacae* Id=37.0% |
| Rz2 | *Pseduomonas cepacia* Id=50.9% |
| | *P. maltophilia* Id=45.4% |
| Rz3 | *Salmonella arizonae* |
| Rz4 | *Klebsiella pneumonia spp* Id=99% |
| Rz5 | *E. colivulneris* Id=60% |
| | *Klebsiella pneumonia* Id=38% |
| Rz6 | *Pseudomonas capacia* Id=97% |
| Rz7 | *Pseudomonas capacia* Id=61% |
| | *Pseudomonas matophilia* Id=31% |

**Table 1:** Most Probable Identification of the Indigenous Bacteria Based on their API Biochemical Profiles

### Molecular identification

The 16S rDNA sequences of the isolates were submitted to Gene Bank sequencing data and aligned against the 16S rDNA sequences of Ribosomal Database project. Table 2 compiles Gene Bank accession numbers of the highest sequence similarity as well as the closest neighbor(s) to the 16S rDNA gene partial sequences of the examined isolates. Sequences of the isolate PS was affiliated to genus Pseudomonas showed 96% similarity to *Pseudomonas stutzeri* M15-10-3. Rz1, Rz3 and Rz6 were closely related to each other and they showed 96% similarity to *Escherichia coli* SCD-1, 95% similarity to *Escherichia* sp. BBDP27 and 96% similarity to Escherichia coli O7:K1 CE10, respectively. RZ4 showed 95% similarity to *Klebsiella* sp. SZH11 while Rz7 showed 99% similarity to *Providencia vermicola* W9B-11a. Rz2, Rz5, and S1 were affiliated according to their 16S rDNA to members of genus Bacillus. They all showed high similarity (97%) to *B. cereus*, *B. amyloliquefaciens* T004 respectively. The phylogenetic relationships of the experimental isolates and closely related species were analyzed using the multisequence alignment program (MEGA 5.1) and the results are presented in phylogenetic tree (Figure 1).

| Similarity % | GenBank accession of the Nearest neighbor | Nearest Neighbor(s) | Isolate No. |
|---|---|---|---|
| 96% | HM576813.1 | *Escherichia coli* SCD-1 | Rz1 |
| 97% | EU621383.1 | *Bacillus cereus* | Rz2 |
| 95% | DQ337505.1 | *Escherichia* sp. BBDP27 | Rz3 |
| 95% | GU384262.1 | *Klebsiella* sp. SZH11 | Rz4 |
| 97% | HQ840415.1 | *Bacillus amyloliquefaciens* T004 | Rz5 |
| 96% | CP003034.1 | *Escherichia coli* O7:K1CE10 | Rz6 |
| 99% | EM-PRO:HQ238823 | *Providencia vermicola* W9B-11 | Rz7 |
| 97% | HQ840415.1 | *Bacillus amyloliquefaciens* T004 | S1 |
| 96% | HM030751.1 | *Pseudomonas stutzeri* M15-10-3 | PS |

**Table 2:** Similarity Percentages of the Selected Isolates to the Nearest Neighbors

### Preliminary selection of efficient bacterial degraders

The 9 bacterial isolates were subjected to preliminary screening based on visual observations to determine the most efficient for carrying the bioremediation of the contaminated effluent. According to such observations, 4 isolates (Rz6, Rz7, S1 and PS) identified as *Escherichia coli* (Rz6), *Providencia vermicola* (Rz7), *Bacillus* sp. (S1) and *Pseudomonas* sp.(PS) were the best for reducing the wastewater turbidity indicating their ability to degrade the contaminants while the other 5 (R z1-Rz5) showed no ability. Therefore, Rz6, Rz7, S1 and PS were selected for the bioremediation assays.

### Biological treatment using free living bacteria in a batch mode

Wastewater was subjected to treatment in a batch experiment using the four selected bacterial species on individual basis in addition to their mixed culture for one week where samples were drawn at 24 h interval. The following represents residuals and removal efficiencies (RE%) of the selected quality parameters as a result of the biological activity of the selected bacteria at different exposure times.

### pH

No significant variations were recorded in the control or the seeded wastewater before or after the treatment process (data not shown). Untreated effluent was slightly acidic (5.63) at the start point and slightly increased during the treatment reached the highest value of (6.9). The same pattern was recorded with the all seeded effluents.


**Figure 1:** Neighbor-Joining Tree Based on 16S rRNA Sequences of the Indigenous and Exogenous Bacteria. Evolutionary distances were calculated using the Kimura 2 model using MEGA5 software. The numerals show the results of the bootstrap analysis values from 1000 replicates (only bootstrap values above 50% were shown). The sequence in bold was determined in this work

## Dissolved oxygen (DO)

DO levels of the seeded and un-seeded wastewater recorded noticeable variations with time (Data not shown). A remarkable decrease in the raw wastewater DO (3.5 mg/l) was recorded with increasing exposure time compared to the control (un-seeded) wastewater. This is mainly due to increase DO consumption during oxidation of the included organic matter with the addition of bacteria. Among the tested species the mixed culture exhibited the highest biodegradation activity shown as sharp decrease in the DO reaching the lowest level (0.45 mg/l) after 7 exposure days. This was followed by Rz7, PS, Rz6 and finally S1 that reached final DO levels of 0.50, 0.85, 1.10 and 1.20 mg/l, respectively at the end of the experiment indicating their variable biodegradative capabilities. The absence of remarkable change of DO in the control effluent indicates poor ability of the wastewater-inhabiting bacteria for biodegradation and thus oxygen consumption.

## Total dissolved solids (TDS)

TDS increased due to remediation process and formation of simple dissolved solids resulting from breaking down of complex contaminants (Figure 2). TDS levels in all wastewater samples either seeded or not showed general trend of increasing with increasing exposure time (till the 7$^{th}$ day) with very few exceptions and clear variations among the tested species. Raw wastewater recorded 945 mg TDS/l at zero time which increased in the seeded wastewater in the range 0.7 and 10.1% based on the ability of the tested species to degrade and/or transform the available contaminants in wastewater. The mixed culture was the most active strain resulted in 1040 mg/l with 10.1% increase in the dissolved salts level after the 7$^{th}$ exposure day. This was followed by the S1, Rz7, Rz6 and finally PS with salt addition ranged between 3.4 and 7.9% also after the same time.

Concerning salt removal, some of the tested species showed salt removal especially at the beginning of the treatment process which were quickly shifted to add salts as a result of contaminants transformation. In that regard, RE of the TDS ranged between 0.5% (S1 after 4 days) and 14.3% (Rz7 after one day). However, control wastewater showed lower SA (0.5-2.1%) compared to the seeded samples while it showed also 4.8% RE after 4 days confirming low biodegradation activity.

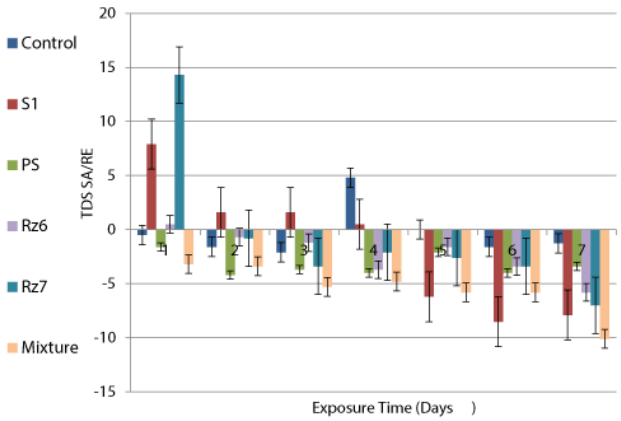


**Figure 2:** Removal or Addition (RE or SA %) of TDS in the Raw and Treated Domestic Wastewater at Different Exposure Times

## Total suspended solids (TSS)

As a general pattern for all treatments, the removal efficiency was increased with time regardless seeded or un-seeded effluents (Figure 3A). Raw wastewater recorded high level content (2300 mg/l), which is generally decreased to the lowest level (1400 mg/l) after 7 treatment days by the most active strain S1 representing RE of 39.1%. This was followed by Rz6, PS, control, Rz7 and finally the mixed culture recording 34.8, 30.6, 30.4, 26.1 and 21.7% RE respectively.

## Chemical oxygen demand (COD)

Raw wastewater recorded relatively high COD level (925 mg/l) at the zero time which is proportionally decreased with time in all wastewater samples (seeded and not) reaching the lowest residual concentration (RC) at the last exposure day (Figure 3B). The largest COD removals by all treatments were achieved within the first 24 h of exposure time. Significant variations in the COD removal were recorded among the tested bacteria with PS considered the most active (RE=93.0%) followed by the Rz7 (90.3%), mixed culture (89.2%), S1 (85.6%), Rz6 (80.5%) and finally the control with the lowest achieved efficiency (61.1%) all after 7 days confirming powerful ability of the augmented bacteria.

## Biochemical oxygen demand (BOD)

The recorded BOD level in the raw effluent (440 mg/l) was proportionally decreased with time in all the seeded wastewater samples only reaching the lowest residual concentration (RC) at the last exposure day (Figure 3C). During the first 24 h, four wastewater samples (S1, PS, mixed culture and the control) showed increases in the BOD with different proportions. PS and the mixed culture-wastewaters showed very slight BOD increases (0.4%) while S1-seeded and control wastewaters showed significantly higher increases (9.5 and 11.3%) respectively. These increases reflect toxicity of the wastewater that led to the death of some bacterial cells adding their organic matter to the wastewater contained them. Rz6 and Rz7 represent the most resistant strains followed by PS, the mixed culture and finally S1. Bacteria inhabiting the domestic wastewater represent the most sensitive strains and showed regular death and increase in the BOD with time in the control wastewater reaching the highest level (148.7%) after 7 days.

Removal efficiency of the BOD increased regularly in all the seeded wastewater samples until the last exposure day. PS exhibited the highest RE of BOD followed by the mixed culture, Rz7, Rz6 and finally S1 with the least RE recording 87.5, 78.9, 75.0, 69.8 and 31.9% respectively after 7 days. However, control wastewater did not achieve any removal but exhibited BOD addition (i.e. 2.49 fold of the initial BOD) after the same time.

## Fat, oil and grease (FOG)

FOG in the raw wastewater recorded 2000 mg/l which considered high level. The bulk removals of FOG took place during the first 24 h and gradually decreased with time in all treatments till the 7$^{th}$ exposure day (Figure 4A). Rz6 and Rz7 exhibited the highest RE (90%) followed by the mixed culture, PS and finally S1 (87.1, 85.0 and 75.0%, respectively). However, control wastewater showed only 20% confirming the ability of the bioaugmentation of bacteria.

## Total coliforms (TC) and fecal coliform (FC)

Since the present study deals with domestic wastewater, total and fecal coliform (TC and FC) are the most important quality parameters due to their effect on the receiving water with their pathogenic load. They also determine the efficiency of the treatment process. Elimination or minimization of TC and FC count indicates efficient treatment while increasing TC and FC indicates the opposite. RE of TC and FC (Figure 4B and 4C) were positively correlated with time in all seeded-wastewaters while absolutely no removal was recorded in the control sample confirming the antagonistic effects of the augmented bacteria on the pathogenic species found in the domestic wastewater.

Raw wastewater showed high counts for both TC (1.0-9.6x10$^9$ CFU/ml) and FC (1.0-69.0x10$^8$ CFU/ml). Augmented bacteria showed remarkable ability for TC and FC removal from polluted wastewater. The mixed culture exhibited the highest RE% of TC (99.0%) followed by PS (98.1), Rz7 (95.2) and Rz6 (88.0%) at the 7$^{th}$ day of exposure. The same pattern was recorded for the removal efficiency % of FC with almost 100% removal recorded by the mixed culture and Rz6 at the last day of exposure.

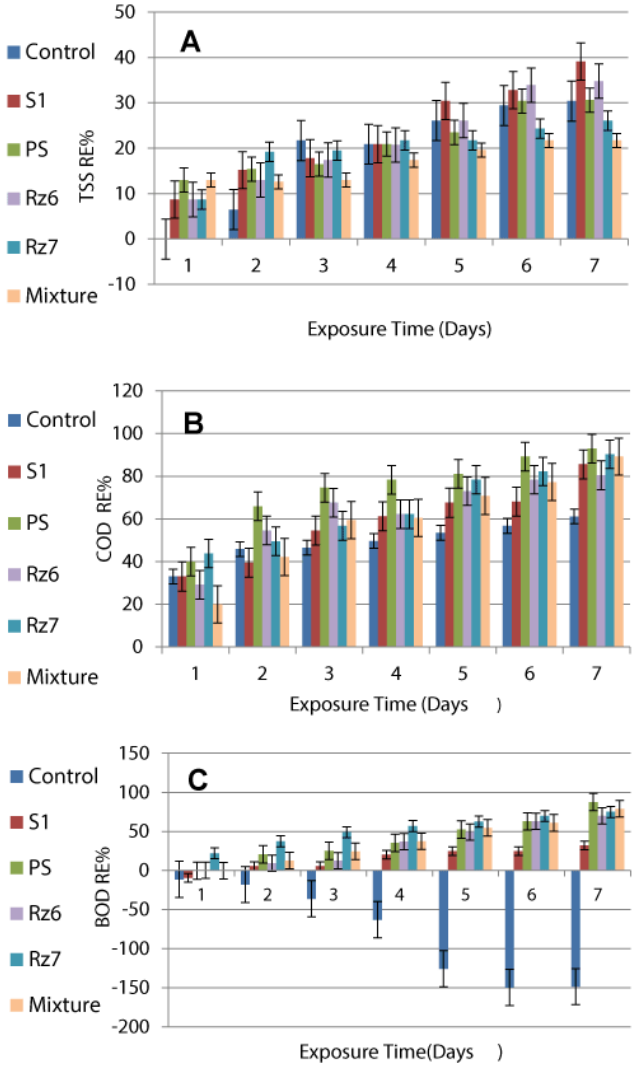


**Figure 3:** Removal Efficiency (RE %) of A) TSS, B) COD and C) BOD in Rawand Treated Domestic Wastewater at Different Exposure Times

However, S1 showed reverse trend where it had no or low antimicrobial effect on TC bacteria while it showed more positive role in the removal of FC with no increases in the FC count indicating that it has selective antagonistic effect on FC only. RE of FC by S1 was fluctuated between a minimum of 38.5% and a maximum of 92.3% confirming the effect of the toxic components on that strain which

directly affected its performance up and down. Control wastewater sample showed the opposite trend with continuous increase in the TC starting from 120% after one day to 760% after 7 days and continuous increase in the FC ranged between a minimum of 300% and maximum of 800% indicating the absence of antagonistic bacteria that can kill and assimilate coliform bacteria.

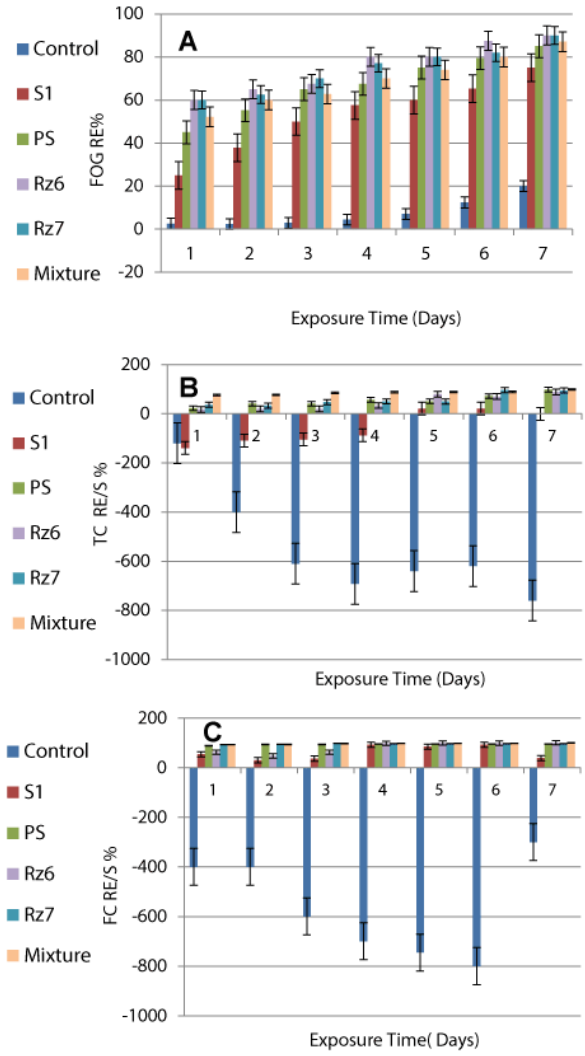


**Figure 4:** Removal Efficiency/Stimulation (RE/S%) of A) FOG, B) TC and C) FC in the Raw and Treated Wastewater at Different Exposure Times

## Discussion

Among the 7 wastewater indigenous isolates only two (Rz6 and Rz7) as well as two exogenous strains (S1 and PS) showed the ability to clarify domestic wastewater indicating that they possess the required degrading enzymes. Molecular characterization of the 4 most active bacteria revealed their belonging to *Escherichia coli* (Rz6), *Providencia vermicola* (Rz7), *Bacillus* sp. (S1) and *Pseudomonas* sp. (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. The marvellous resistance and superior potentiality of the present bacterial selection for biodegradation of toxic organic and uptake of inorganic pollutants were reported earlier [9,10,12,19-21]. *Bacillus* spp. such as Rz5 and S1 are well known as highly resistant spore-forming bacteria that possess excellent characteristics and extremely efficient for many agricultural, environmental and industrial applications [20,22-26]. Results confirmed the great and remarkable ability of the selected Bacillus sp. to degrade all the investigated contaminants at very fast rates which explains their occurrence in the highly contaminated and hypertrophic environments such as Lake Mariut, a brackish water lake in south Alexandria, Egypt.

*Pseudomonas stutzeri* (PS) is a Gram-negative, rod-shaped, motile, single polar-flagellated, soil denitrifying bacterium with superior biodegradation and transformation ability for many environmental pollutants [27]. Active and dead masses of Pseudomonas spp. are well known as biodegraders for pesticides [10,21], crude [9] and vegetable oil [12].

Wastewater treated in the present study can be classified as moderately strong depends on levels of contaminants it contains that required powerful treatment to minimize its pollution load and discharge it safely. Batch treatment using free living individual and mixed selected bacteria aimed to design an efficient treatment process for eliminating or minimizing chemical, biological and organic load from the drainage network in Jeddah City to protect the receiving ecosystem. It was time and species dependent and subsequently resulted in varied removal efficiencies of contaminants.

Total suspended solids (TSS), biological oxygen demand (BOD) and chemical oxygen demand (COD) are three major waste characteristics determining the pollution levels in wastewater. They are also the main identification indicators for determining the efficiency of any proposed treatment system. The maximum permissible limits (MPLs) of these indicators are specified nationally by laws and regulations as those of Egypt and Saudi Arabia to provide a safe discharge. PS considered the most efficient for removing organic matter (BOD and COD) brought them to safe discharge limits in the batch treatment. However, S1, Rz6, Rz7 and the mixed culture showed the highest removal of TSS, FOG, TC and FC respectively but their residuals still above their MPLs for the safe discharge (23.3, 20 and 200 fold for TSS, FOG and TC respectively).

Microbial degradation requires optimum pH and temperature to proceed. In the present study pH range (5.60 to 6.90) and optimum temperature of 37°C encourage all the selected bacteria to remove organic matter and other included pollutant which is supported by other workers [28]. However, pH of the treated domestic effluent lies in the permissible limits (6-9) stated by the Presidency Meteorology and Environment Organization (PME), KSA. Environmental laws stated that DO content of water and wastewater should not decrease below 5 mg/l to be safely discharged. Raw wastewater recorded 3.5 mg/l DO that is lower than DO MPL (5 mg/l). More oxygen is required for degradation of the included organic matter to the extent that wastewater may be depleted of oxygen during stabilization. Therefore, high amounts of oxygen in addition to powerful bacterial strains are needed for degradation of the contaminants. Total suspended solids TSS include settle able and colloidal fractions. The colloidal fraction cannot be removed by settling. Generally, biological oxidation or coagulation, followed by sedimentation, is required to remove these particles from suspension. The highest TSS RE recorded

was 39.1% achieved using the free living *Bacillus amyloliquefaciens* (S1) after 7 day which is 23.3 fold higher than its maximum permissible limit (MPL) of 60.

*Pseudomonas stutzeri* (PS) achieved the highest RE of BOD (87.5%) after 7 days produced an effluent with good quality (55 mg BOD/l) that can safely be discharged into open systems. BOD is removed mainly by the powerful ability of the augmented bacteria that metabolize organic matter found in wastewater transforming them into harmless by-products such as carbon dioxide and water. During the first 24 h, BOD of wastewater augmented with *B. amyloliquefaciens*, *P. stutzeri* and the mixed culture as well as the control increased at varies levels (9.5, 0.4, 0.4 and 11.3% respectively). The control continued to have higher BOD reaching the highest increase (-148.7%) after 7 exposure days. These increases reflect toxicity of the wastewater that led to the death of some bacterial cells with the addition of their organic matter to the wastewater as BOD. It is also reported that unless the microbial cells produced during organic matter decomposition are removed from the solution, complete treatment will not be accomplished because biomass of these cells will be measured as BOD in the effluent. In such case the only treatment that has been achieved is that associated with the bacterial conversion of a portion of the organic matter originally present to various gaseous ends by products [29].

Raw wastewater recorded relatively high COD level (925 mg/l) at the zero time that was regularly decreased till the $7^{th}$ exposure day reaching 65 mg/l (93.0% RE) achieved by *Pseudomonas stutzeri* (PS). The efficiency of *P. stutzeri* (PS) in removing COD load is also supported by Ramteke et al. [30] for heavily polluted tannery effluents. According to the MPL of the COD (100 mg/l in the Egyptian regulations) the lowest recorded RC (65 mg/l) of the COD is much lower and compiles with the law for safe discharging into open environments.

Raw wastewater recorded very high FOG level (2000 mg/l) at the zero time that was regularly decreased till the $7^{th}$ exposure day reaching 200 mg/l (90.0% RE) achieved by Escherichia coli (RZ6) indicating high capability of the selected bacteria to use fatty organic matter as a source of carbon and energy [11]. The lowest FOG residue recorded 200 mg/l which is 20 fold higher than the MPL of FOG in the final treated effluent (10 mg/l Egyptian Limits) and 1.7 fold higher than Saudi Limit (120 mg/l) stated for the discharge into Central Treatment Unit [31,32].

Toxicity of wastewater on the selected bacteria was determined as growth inhibition (GI) shown in the total viable count of bacteria (TVC). GI gradually increased reaching the highest GI after $7^{th}$ day. In that respect, mixed culture recorded the highest (99.1%) growth inhibition after the $7^{th}$ day. Control wastewater showed relatively lower GI (75% after 7 days) compared to the seeded wastewater. These results are attributed to higher toxicity of wastewater posed on the exogenous bacteria that deal with the included contaminants compared to indigenous bacteria that are inactive towards those contaminants. However, bacteria survived, although of low densities, acquired high resistance against wastewater toxic pollutants [33,34].

Raw wastewater contains huge amount of total coliform (TC) ($1.0\text{-}9.6\times10^9$ CFU/ml). Augmented bacteria showed remarkable ability for TC removal from polluted wastewater. Again, the mixed culture exhibited the highest RE% of TC (99.0%). In contrast control sample showed the opposite trend with continuous increase in the TC starting from 120% after one day to 760% after 7 days indicating the absence of antagonistic bacteria that can kill and assimilate coliform bacteria [35,36]. Also, very high fecal coliform (FC) density ($1.0\text{-}6.9\times10^9$ CFU/ml) was recorded in the raw wastewater. However, augmented bacteria showed remarkable ability for FC removal from polluted wastewater with the mixed culture exhibited the highest (almost 100%) removal of FC. Control wastewater FC kept the same trend as for the TC where it recorded continuous increase in the FC ranged between a minimum of 300% and maximum of 800% due to the absence of antagonistic bacteria [37]. TC that includes both fecal and non-fecal coliform in the treated sample was slightly higher ($0.1\times10^9$ CFU/ml or $1\times10^8$, i.e. $1000\times10^5$ CFU/ml) which means 200 fold higher than the MPL of the TC. However, such increase is mainly composed of non-pathogenic coliform bacteria which represents much lower environmental risk and can be easily removed by the traditional chlorination in a subsequent disinfection stage.

Results of the present study confirmed the ability of the selected bacteria for the removal of the target contaminants including antagonistic effect against pathogenic bacteria (coliform) and thus can be manipulated efficiently to decontaminate polluted systems providing the optimum degradation conditions. Removal of such contaminants was controlled by microbial species, concentration of pollutants in the tested wastewater and finally the contact time between wastewater and the bacterial cells.

## Conclusion

In conclusion, the batch treatment of the contaminated domestic effluent was time - and bacterial species-dependent. PS considered the most efficient for removing organic matter (BOD and COD) while mixed culture considered the most efficient for removing fecal coliform (≈100%) brought them to safe (60, 100 mg/l and ≈ 0.0 CFU/ml respectively) discharge limits (MPL) stated by the Egyptian (Laws no. 48/1982 and 4/1994) and Saudi Environmental laws that regulate discharging of domestic and industrial wastewater into fresh and saline open water. In addition, high removals of TSS, FOG and TC (39.1, 90.0 and 99.0) were achieved by *B. amyloliquefaciens* (S1), *E. coli* (Rz6) and the mixed culture respectively. However, their residuals still higher (23.3, 20 and 200 fold respectively) than their MPLs for the safe discharge due to the short treatment course. Therefore, longer treatment time and/or using fixed forms of the selected bacteria are highly recommended to bring the contaminated domestic effluent it to the safe limits for the environment.

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