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Biodegradation of municipal wastewater with local and commercial bacteria

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Abstract

This study was undertaken to evaluate the degradation of synthetic and real municipal wastewater by commercial bacteria known as SludgeHammer bacteria and some local isolated strains. TOC removal efficiencies in the synthetic wastewater were 70%, 54%, 52%, 42% for the SludgeHammer, *B. subtilis*, *B. laterosponus* and *P. aeruginosa*, respectively. TOC degradation experiments using municipal wastewater found that the mixed bacteria culture of selected strain of *B. subitlis* and *P. aeruginosa* increased treatment performance by 6 to 16% higher than other strains. However, they took relatively longer time to reach the maximum degradation rate compared to the SludgeHammer bacteria.

Keywords: Bioaugmentation; synthetic wastewater; municipal wastewater; SludgeHammer bacteria

1. Introduction

Allowing municipal wastewater to be released to the environment can make a lot of problems. The wastewater may be treated by physicochemical or biological processes. Biological treatment is preferred solution for improving the municipal wastewater treatment. Recently the biological process is the main treatment system in municipal wastewater plant. The performance of a biological process is often enhanced through bioaugmentation of one or more species of specialized microorganisms (Semrany et al. 2012, Erdogan et al. 2011, Karamalidis et al. 2010).

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The microorganisms usually bacteria are selected on the basis of accelerated rates and their ability to degrade specific compounds. Many researcher have reported that introduce of indigenous strains of bacteria can enhance the degradation of organic pollutants in wastewaters. Qu et al. (2011) have found that *pseudomonas spp*. (isolated from activated sludge) was very effective in removing phenol from industrial wastewater compared to the non-augmented one. Tuo et al. (2011) found *Bacillus spp*. have high ability for quinoline removal. Other work by Chen et al. (2009) investigated the treatment of municipal wastewater with single strain of bacteria and bacterial consortium and found that bioaugmentation with three strains of *E. cloacae, Gordonia* and *P. putida* would be more effective in removing TOC compared with that of single strain treatments. Similar results by Mongkolthanaruk et al. (2002) found that treatment of lipid-rich wastewater by a mixed culture *of pseudomonas aeruginosa, Bacillus sp.* and *Acinetobacter calcoaceticus* reduced the BOD and lipid content from 3600 mg/l and 21,000 mg/l, respectively, to less than 20 mg/l within 12 days. Moreover, these 3 strains were more effective in treatment of wastewater when compared to a single strain treatment. In other study by Schneider and Topalova (2011) reported that municipal wastewater treatment inoculums was as efficient as commercial inoculums (namely: hydropacks; Bilikuk and Laktazyme), in removing the COD and phenols from dairy wastewater. This work aims to compare the bioaugmentation of municipal wastewater with indigenous and commercial bacterial strains.

2. Methods

2.1. Sample collection

Sewage water samples were collected from different sewage water treatment station in Tripoli. The sample were collected in sterile conical flask from the aeration thank, by using sampler and kept in refrigerator for future use.

2.2. Isolation, identification and preparation of inoculums

Isolation of bacteria was done by using serial dilution method. 0.1ml from each dilution bottle was streak on Nutrient agar plates by spread method. The Petri dishes were incubated aerobically at 37 °C until colonies have appeared. The isolates were re-cultured on new media for purification to get a single pure strain. Gram stain was performed for each isolate. Biochemical tests were studied by using standard biochemical assays according to the scheme Berge's manual and confirmed by Analytical Profile Index 20(API 20E) system technique. To obtain the appropriate biomass of bacterial isolates, the isolates were inoculated separately in individual Erlenmeyer flask contain nutrient broth medium and were incubated for 24h at 37°C in the rotary shaker incubator. The cells harvested by using centrifugation at 4000 rpm for 10 min and the supernatant was discards. The cell concentration of each strain measured using spectrophotometer at 600nm (OD600) in order to obtain a 0.5mcf or $1.5x10^8$ cfu/ml.

3. Biodegradation Experiments

3.1. Media preparation

A synthetic wastewater containing the following compositions was prepared as described by Chen (2009): (g/l) 7.5 glucose, 1.15 NH_4Cl, 0.2 KH_2PO_4, 0.2 g $MgSO_47H_2O$, 0.25 $CaCl_2$. The solution diluted to a desirable concentration of TOC 220mg/l and used as the synthetic wastewater in the single strain. 0.5 ml of the trace element solution was added for this medium with the following composition: (mg/l) H_3BO_3(50), $ZnCL_2$ (50), $CuCL_2$ (30), $MnSO_4$(500), $COCL_2.H_2O$(30), $(NH_4)6MOO24H_2O$(50). The pH was adjusted to 7.1 and the media was autoclaved at 121°C for 15 minutes.

3.2. Experiment study

Commercial strain of SludgeHammer and local isolated strains of bacteria identified as *Bacillus spp*., *Bacillus subtilis*, *Bacillus laterosponus*, *Bacillus agri*, *Pseudomonas aeruginosa*, *and Pseudomonas putida* were used.

10ml of each strain was added as inoculums in 500ml flask contain 240ml synthetic wastewater, the flasks were incubated in the rotary shaker at 170rpm at 37°C . Samples were collected ever two hours to monitor bacterial growth at 600nm and biodegradation rates. The bacterial growth and biodegradation rates were monitoring by measuring the optical density and TOC.

3.3. TOC Determination

TOC analysis were carried out using Hach Lange Cuvette test (LCK 386) as follows: 1.0ml of sample was pipetted into the digestion cuvette, which then inserted open in the TOC-X5 shaker for five minutes. When the sample preparation is completed, the indicator cuvette with membrane double cap was tightly screwed on the TOC cuvette, and then digested at 100 °C for 120 min, then allowed to cool, cleaned and evaluated

4. Results and discussion

4.1. Determination of bacterial growth and biodegradation using a single Strain

The ability of an individual strain of bacterium to treat a wastewater was monitored by measuring both the cell growth and biodegradation of TOC in a synthetic wastewater samples. All the samples (120ml with 220mg/l TOC) were inoculated with 10ml of active strain and gave initial optical density varying from 0.037-0.056 optical density (OD600) of bacteria. Samples were withdrawn every 2 hours, and the OD600 and TOC were measured. Fig. 1 and 2 show the growth of bacteria and the residual TOC concentration, respectively. The growth phase patterns (Fig.1) demonstrate that all the local bacteria took longer time to reach stationary phase compared to the SludgeHammer bacteria.

To evaluate the performance of each strain in degrading TOC, the growth kinetic parameters were determined and compared in Table 1.

Table 1. The growth kinetic parameters.

Parameters	*B. agri*	*B. Subtilis*	*B.Later-osponus*	*B. spp*	*P.Putida*	*P.aeruginosa*	S-Hammer
T_{lag}(h)	2	2.5	1	4	2	1	1
$L_{initial}$	0.044	0.04	0.056	0.052	0.049	0.037	0.043
L_{max}	0.074	0.08	0.091	0.063	0.095	0.077	0.12
Ts (h)	14	12.5	14	12	12	10.5	7
μ_{max} (h-1)	0.063	0.139	0.046	0.026	0.095	0.098	0.286
r_{max} (%)	36.57	54.37	52.34	32.41	25.88	42.57	71.25
q_{max} mgTOC/(d-mgVSS)	0.423	0.021	0.393	0.026	0.522	0.590	1.489

Lmax: the maximum cell density, Ts: the time required for getting stable phase of TOC degradation, rmax: the maximum TOC removal efficiency, μmax: the specific growth rate of cell, and qmax: the maximum specific substrate utilization rate.

Among the six natural strains*, B. subtilis, B. laterosponus, P. aeruginosa* had relatively higher TOC degradation ability with r_{max} of 54%, 52%, and 43%; respectively. However, *Pseudomonas aeruginosa* had slightly greater q_{max} (0.59 mgTOC/(d-mgVSS) and shorter Ts (10h). These results revealed that *Pseudomonas aeruginosa* was the most effective strain for degrading the organic substrate (glucose) in the synthetic wastewater. However, the natural local strains showed much less degradation (20-40% less) when compared to the SludgeHammer bacteria (see Fig 2). From the kinetic parameters in Table 1, this bacteria had the greatest r_{max} (71%) and the shortest T_{lag} (<2h) and highest q_{max} (1.5 mgTOC/(d-mgVSS). Using these parameters, it is hypnotized that the “SludgeHammer” will improve the treatment performance of municipal wastewater.

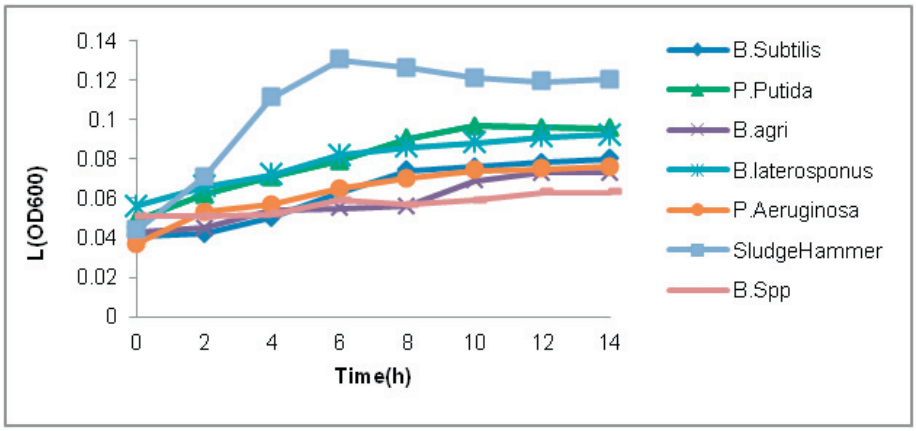

Fig. 1. Cell growth of each bacterial strain in synthetic wastewater.

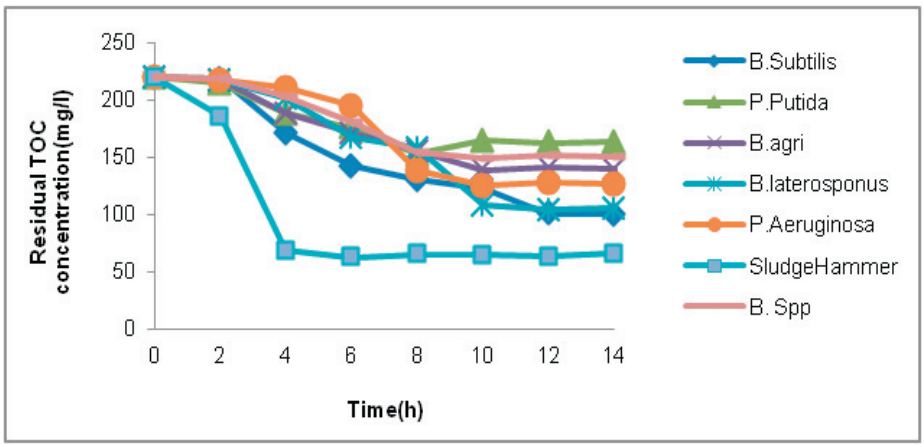

Fig. 2. Biodegradation of synthetic wastewater by each bacterial strain.

4.2. Bioaugmentation of the selected strains on municipal wastewater treatment

Considering the significant effect obtained from *B. Subtilis* (R1) and *P. Aeruginosa* (R2) and the "SludgeHammer" (R3) for degrading TOC in the synthetic wastewater, the three strains were selected for additional degradation experiment using raw municipal wastewater. The strains were added (4% inoculums has 10^8CFU/ml) as a single strain of R1, R2, or R3 and a combined strain of R1+R2. The controlled bioreactor was operated without bioaugmentation. As can be seen in Fig. 3, the removal efficiency of TOC in the bioaugmented reactors were clearly better (6 -16% higher) than controlled reactor. The reactor inoculated with the selected combined strains (R1+R2) yielded relatively better treatment performance compared to the reactor inoculated with single strain of either R1, R2, and R3.

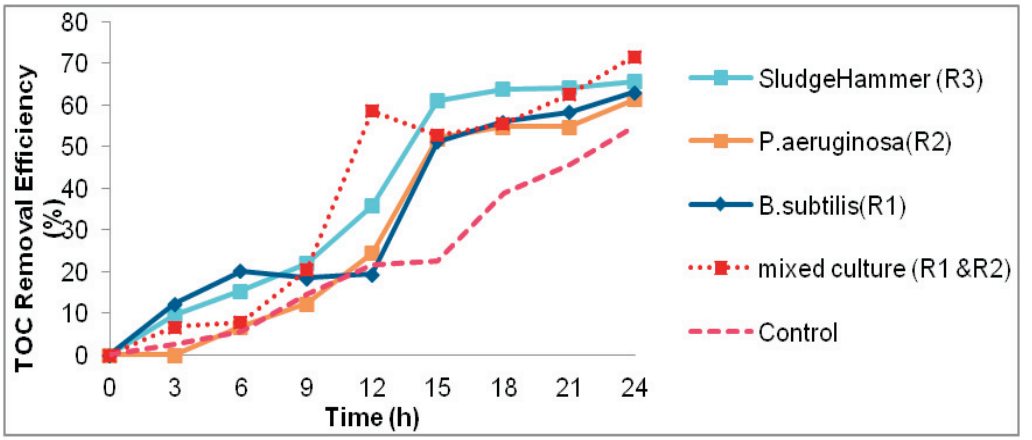

Fig. 3. TOC removal efficiency in bioaugmented and control reactors

5. Conclusions

Biodegradation of municipal wastewater by a mixed bacterial culture of *P. aeruginosa* and *B. Subtilis* were relatively better than biodegradation by a single bacterium. The mixed culture, however, it took longer time to reach their maximum removal than SludgeHammer bacteria. This study suggests bioaugmentation of wastewater treatment reactor with highly specialized strains can improve the treatment.

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