Assessment the ability of some bacteria and actinomycetes strains in the treatment of sewage sludge

Ibraheem I. B. M[1], Hammouda, O[1,] Abdel-Raouf, N[1], Abdel-Tawab, M.S[2] and Faysal, A[2]

[1] Botany and Microbiology Department, Faculty of Science, Beni-Suef University, Beni-Suef, 62514, Egypt.
[2] Beni-Suef Wastewater Treatment Plant, Beni-Suef, Egypt
*E-mail: faysalamira@yahoo.com

Abstract; The current study aimed to assessment the ability of the previously isolated eight strains of bacteria and actinomycetes in the biodegradation and removal of the organic pollutants of sewage sludge (SS) through using the dried sludge (DS) of Wastewater Treatment Plant, Beni-Suif City, Egypt as a substrate media and measuring the removal efficiency of COD, BOD, TSS and VSS. Different concentrations of DS 1, 2, 3 and 4gm in different conditions of aerobic and anaerobic treatment were applied. Three grams of concentrated DS in anaerobic conditions were measured high removal efficiency reached 78, 80, 60 and 52% for COD, BOD, TSS and VSS respectively with *Nocardiopsis lucentensis* and 74, 86, 55 and 43% for COD, BOD, TSS and VSS, respectively with *Saccharomonospora azurea*. Also mixture of the two potent actinomycetes (*N lucentensis* and *S azurea*) measured highly removal efficiency reached 78, 92, 65 and 63% for COD, BOD, TSS and VSS respectively under normal anaerobic condition (nonsterilized sludge) after 5 days incubation at 32 ^{o} C.

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1. Introduction

Sewage sludge is now becoming a worldwide environmental problem because of its increasing production and its high contents of organic waste and pathogens, also heavy metals and xenobiotics (Elsayed et al., 2017). The activated sludge method is used as a biological sewage treatment process worldwide; however, there remain several technical issues, including the production of a huge amount of sewage sludge (Liu and Tay 2001). For instance, approximately 620 million and 170 million tons (dry weight) of sewage sludge containing DS are produced annually in United States and Japan, respectively, most of which is incinerated as useless material (Dufreche et al., 2007; Ministry of the Environment Japan 2012). Because the lack of final disposal sites has become a serious problem recently, it is imperative to reduce the amount of DS requiring disposal by making use of it.

It is believed that sewage sludge is a nutrient rich which if processed can be utilized as a source of growth facilitator for microorganisms to enable them carry out their metabolic activities. SS is generally composed of urine, soapy material and household waste and other suspended particles. These wastes are mostly laden with products that serve as source of nitrogen, phosphorus and potassium (Kamaluddeen et al., 2016). Thus, Ferrer et al. (2011) reported that recycling of sewage sludge to agricultural land as part of an integrated farm management plan is recognized as one of the best practicable environmental options for the final disposal of it. Also, Shaheen et al. (2017) observed an improvement in soil fertility and an increase of NPK content after application of organic waste treated by microorganisms to soil.

Gotvajn et al. (2009) has reported that the biological methods are usually preferred over the physicochemical in removing the majority of pollutants in wastewater treatment. Nitrogen and organic pollution act as nutrient substrate for the purifying biomass in biological method. The goals of this method are to reduce labour time, enhance BOD/COD removal, degrade wide range of organic waste, increase the system efficiency, reduce sludge build up and reduce hydrogen sulphide cost. Also from the advantages of using biological systems is that, operation takes place at ambient temperature. This can help in saving the energy consumption (Ishak et al., 2011). There are numerous microorganisms including bacteria, fungi and yeasts are known for their ability to degrade hydrocarbons (Thangaraj et al., 2007; Morelli et al., 2005; Van Hamme et al., 2003; Chaillan et al., 2004). The aim of the present work was to test the ability of some bacteria and actinomycetes strains in the treatment of sludge in the wastewater treatment plant at Beni-Suef, a city in the middle Egypt.

2. Materials and methods

Preparation of microorganisms inoculum for treatment of sewage sludge:

The inoculum of bactria and actinomycetes used in the assessment were previously isolated from drying beds (DS) and from the outlet after thickener tanks (WS) of Wastewater Treatment Plant (WWTP) in Beni-Suef-Egypt. They also purified and identified morphologically, biochemically and by 16S (phylogenic tree). The inoculum was prepared by growing the tested bacteria on nutrient agar for 24 h. A full loop was taken from these plates and grown in 250ml conical flasks containing 50 ml of sterile nutrient broth for another 24 h at 32^{o}C in shaker incubator (120 rpm). The bacterial growth was assessed by colony count to adjust the number of cells to 1×10^{8} CFU. Specific volumes of inoculum were poured into a centrifuge tube under aseptic condition and were centrifuged for 10 minutes at 3000 rpm. The pellets of a known volume of inoculum were re-suspended in 250 ml conical flasks containing 100 ml of sterile SS solution, the relative volume of inoculum was 5% from pure or mixed cultures.

Assessment the ability of the isolated strains in the treatment of sludge (preliminary test):

Different concentrations of DS solution were prepared by dissolving 1,2,3 and 4 gm respectively of (DS) in 100 ml distilled water in 250 ml conical flask for each isolate alone, and autoclaved for 20 mint at 121 °C and 1.5 atm. After cooling, known volume of each isolate inoculated in each flask and incubated at 32 °C for 5 days. Determined the ability of each isolate in degradation of DS by measuring COD, BOD, TSS and VSS. The concentration of DS and isolate which measure high removal percent in biodegradation were selected, at different conditions aerobic (sterile & non sterile) and anaerobic (sterile & non sterile). The best isolates mixed together and testing its ability in the treatment by measuring COD, BOD, TSS and VSS removal at aerobic (sterile & non sterile) and an aerobic conditions (sterile & non sterile).

Chemical analysis

All samples of tested DS were analyzed for the following physico-chemical characteristics: chemical oxygen demand (COD), biochemical oxygen demand (BOD_5), total suspended solids (TSS), and volatile suspended solids (VSS), according to Standard Methods for the Examination of Water and Wastewater (APHA 2011).

Statistical analysis:

The obtained results were statistically analyzed by one way analysis of variance (ANOVA) to determine the degree of significance between the treatments using SPSS 16 software. Significance was set at $p < 0.05$.

3. Results:

The data in Figures 1, 2, 3 and 4 indicated that the 4 gm concentration of DS added as substrate for testing the biodegradation efficiency of isolated strains was inhibitor for all tested isolated strains. On the other hand 3 gm concentration of DS record the best result, where it obvious from Figures that most isolates specially *Saccharomonospora azurea* can degrade the organic matter in DS with highest removal efficiency reached 60 %, 49% 58% and 48% for COD, TSS, BOD and VSS measurement parameters, respectively followed by *Nocardiopsis lucentensis* which also record high removal and biodegradation efficiency reached to 58%, 45 %,48 % and 41%for COD, TSS, BOD and VSS measurement parameter after 5day incubation at room temperature. It was clearly noticed also from Table 1, that *Saccharomonospora azurea* record the highest ability to degrade and treat DS as all measured parameter reached to78, 86, 55 and 43% removal efficiency for COD, BOD, TSS and VSS respectively under normal anaerobic condition without sterilization for DS nearest to it *Nocardiopsis lucentensis* which record 74, 80, 60 and 52% removal efficiency in the measurement parameters also under normal anaerobic condition without sterilization for DS at 32°C compared to aerobic and sterilized conditions.

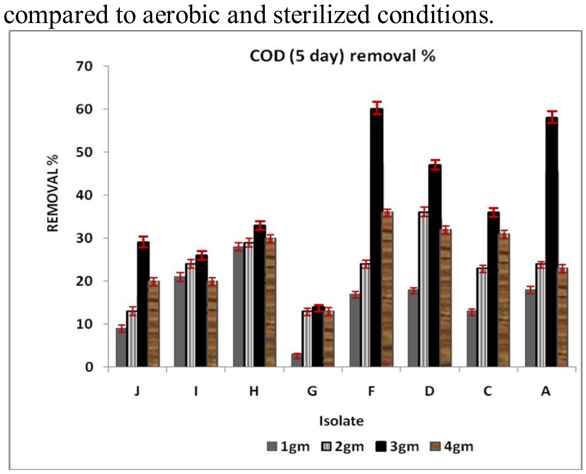

Figure 1. COD removal efficiency at different concentrations of DS

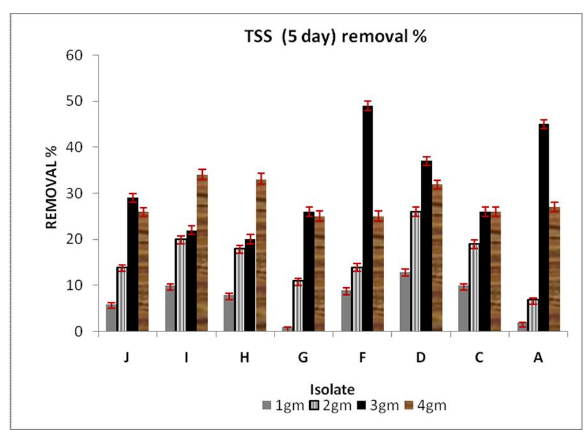

Figure 2. TSS removal efficiency at different concentrations of DS

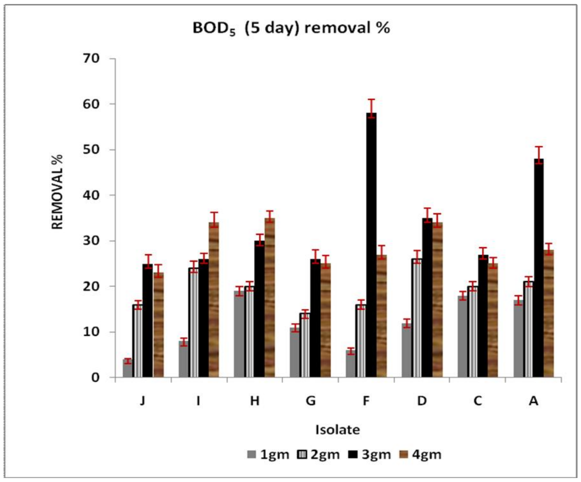

Figure 3. BOD_5 removal efficiency at different concentrations of D

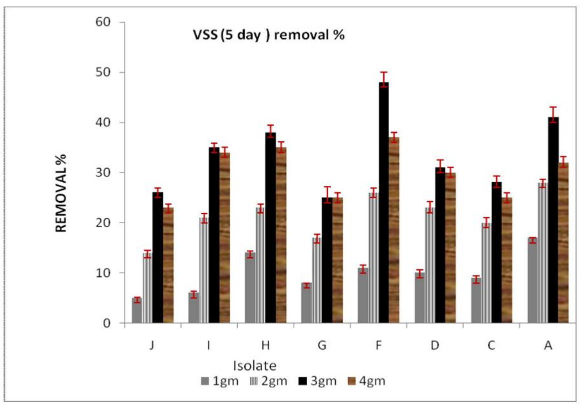

Figure 4. VSS removal efficiency at different concentrations of DS

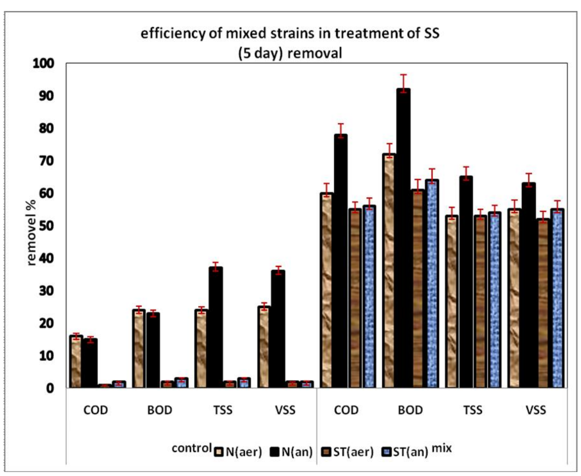

Figure 5. The efficiency of the mixed culture in the treatment of DS (non sterilized and sterilized) under aerobic and anaerobic condition.

Assessment the efficiency of the mixed culture in the treatment of sewage sludge (non sterile and sterile) under aerobic and anaerobic condition: Confirmed test.

The above data indicated that strains *Saccharomonospora azurea* and *Nocardiopsis lucentensis* were the most potent strains in the treatment of DS so two cultures were chosen as single culture and mixed together to assimilate its ability in the treatment under aerobic and anaerobic condition at 32°C for 5 days and compared to control. It was obvious from the results in figure 5. That mixing of (*N lucentensis* and *S azurea*) cultures attained the best removal percentages reached 78, 92 % 65% and 63% for COD, BOD, TSS and VSS respectively under normal anaerobic conditions.

4. Discussion

The data mentioned above showed that most tested organisms achieved the reduction in all measured parameters especially at normal anaerobic conditions compared to control. Sewage bacteria have been used by (Watson and Jones 1977) to degrade Polyethylene. Azab (2000) observed that the important parameter of wastewater e.g. COD and BOD were improved by 24.13% to 66.92% depending on the organism and the culture conditions. Also, Arquiaga et al. (1995) stated that the biological treatment is an important means by which toxic or hazardous organic compounds can be economically converted to less noxious materials.

The data also improved that the two potant actinomycetes have a high removal efficiency specially under anaerobic and mesophilic conditions reached 78, 80, 60 and 52% for COD, BOD, TSS and VSS respectively with *Nocardiopsis lucentensis* and 74, 86, 55, and 43% for COD, BOD, TSS and VSS, respectively with *Saccharomonospora azurea*. Hozzein et al. (2011) isolated actionomycetes strains such *Nocardia* and *Streptomyces* from wastewater and record their high removal efficiency of pollutants.

The results above are in agree with ASIA et al. (2006) which reported the reduction in solids reached 68% TSS and 51% VSS under anaerobic treatment at mesophilic condition. This indicates that anaerobic system is therefore a good method of reducing the quantity of sludge before disposal. The reduction in the amount of sludge solids here compares favorably also with 60-62% VS reduction by Dinsdale et al. (1996) when they separately treated coffee waste by anaerobic mesophilic digestion. The reduction also in BOD and COD measuring parameters are in agree with (ASIA et al., 2006) which recoded that BOD and COD reductions of 89% each were achieved.

The use of mixed culture provides several advantages over a pure culture. The mixed culture can better adapt to changing conditions during growth. It is clearly noticed that the mixed culture of two actinomycetes give the high removal efficiency of organic and inorganic pollutants reached 78, 92, 65 and 63% for COD, BOD, TSS and VSS parameters under normal anaerobic condition after 5 days incubation at 32°C. It is in agree with Omar (2012) which used mixture of *Pseudomonas putida, Pseudomonas flouresence and Azotobacter vinelandii* in degrading phenols and production of organic biofertilizer from olive mill wastewater.

Table1. The efficiency of the isolated strains in the treatment of sterilized and non sterilized DS under aerobic and anaerobic conditions.

Isolate	*Parameter*	*Nonsterile (anaerobic)*	*Nonsterile (aerobic)*	*Sterile (anaerobic)*	*Sterile (aerobic)*
Control	*COD removal %*	*16*	*15*	*1*	*2*
	BOD removal %	*24*	*23*	*2*	*3*
	TSS removal %	*24*	*37*	*2*	*3*
	VSS removal %	*25*	*36*	*2*	*2*
Saccharomonospora azurea (F)	*COD removal %*	<u>*78*</u>	*33*	*45*	*14*
	BOD removal %	<u>*86*</u>	*44*	*47*	*27*
	TSS removal %	<u>*55*</u>	*32*	*41*	*22*
	VSS removal %	<u>*43*</u>	*32*	*32*	*24*
Kocuria rosea (I)	*COD removal %*	*63*	*25*	*18*	*14*
	BOD removal %	*77*	*34*	*22*	*20*
	TSS removal %	*44*	*30*	*25*	*27*
	VSS removal %	*45*	*29*	*24*	*26*
Nocardiopsis lucentensis (A)	*COD removal %*	<u>*74*</u>	*19*	*36*	*15*
	BOD removal %	<u>*80*</u>	*44*	*43*	*27*
	TSS removal %	<u>*60*</u>	*34*	*37*	*24*
	VSS removal %	<u>*52*</u>	*34*	*22*	*27*
Streptomyces (H)	*COD removal %*	*54*	*35*	*30*	*21*
	BOD removal %	*60*	*40*	*33*	*23*
	TSS removal %	*34*	*24*	*20*	*12*
	VSS removal %	*35*	*26*	*15*	*12*
Streptococcus parasanguinis (G)	*COD removal %*	*64*	*63*	*16*	*13*
	BOD removal %	*70*	*68*	*23*	*22*
	TSS removal %	*50*	*42*	*27*	*22*
	VSS removal %	*52*	*44*	*22*	*21*
Nocardia (J)	*COD removal %*	*64*	*54*	*23*	*20*
	BOD removal %	*72*	*65*	*33*	*26*
	TSS removal %	*44*	*33*	*24*	*20*
	VSS removal %	*45*	*36*	*22*	*15*
Kocuria varians (C)	*COD removal %*	*67*	*52*	*30*	*17*
	BOD removal %	*78*	*68*	*40*	*32*
	TSS removal %	*49*	*33*	*23*	*20*
	VSS removal %	*47*	*34*	*21*	*13*
Dermacoccus nishinomiyaensis (D)	*COD removal %*	*60*	*59*	*22*	*12*
	BOD removal %	*78*	*66*	*33*	*22*
	TSS removal %	*45*	*33*	*21*	*10*
	VSS removal %	*44*	*32*	*23*	*11*

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