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Evaluation of Lyophilization Effects on Operational Parameters and Characteristics of Activated Sludge

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ABSTRACT

Advances in microbial and biotechnology have given great impetus to the filed of pollution control. In some countries efforts are being focused on the application of biotechnology for wastewater treatment. Occurrence of microbial growth inhibitors in the biological reactor will affect their activity, which this in turn will lead to reduced performance of the system in pollutants removal. Restart up of the system, especially in the biological treatment of industrial wastewater, will require abundance time and cost. An effective solution to reduce these problems is to prepare and store dried concentrated sludge bearing the needed microorganisms to be used later. The most effective technique to produce such concentrated sludge is lyophilization in which a stabilized biological solid is produced through rapid freezing and then drying under high vacuum conditions. This study was carried out to investigate the lyophilization effect on the microbial quality of the activated sludge. In this regard, operational characteristics including OUR, SVI, soluble COD removal efficiency and predominant microorganisms species before and after lyophilization in a lab-scale experiment were used. The results showed that sludge lyophilization had no influence on microorganisms' performance and operational characteristics of the activated sludge. In addition, there was not a significant change in the sludge properties before and after lyophilization. Sludge microorganisms were viable after lyophilization. So, lyophilization was concluded to be a suitable technology for preparation and preservation of cells in activated sludge because of preserving viability, ease of handling and simplicity.

Keywords: *Activated sludge, Operational parameters, Lyophilization*

INTRODUCTION

Advances in microbial genetics and biotechnology have given great impetus to the field of pollution control. In some countries efforts are being focused on the application of biotechnology to wastewater treatment (Bitton, 1999). Currently, the application of biotechnology, especially activated sludge, is more widespread due to its many advantages over other wastewater treatment operations.

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In such systems microorganisms play the key role in treatment and removal of pollution; as a consequence, occurrence of inappropriate environmental conditions such as dissolved oxygen, pH as well as toxics and inhibitors presence can influence microbial activity. This in turn would lead to reduced performance of the system. On the other hand, especially in industrial wastewaters which contain more specific chemicals, it may be needed to introduce one or several specific microorganisms not founded in the activated sludge, depending on the existing pollutant (Arceivala, 1998).

Also, biological wastewater treatment, especially for industrial wastewaters, such as activated sludge will require consider. Able time and cost in order to resume as well as enhance the system start-up (if it collapses) because production of sludge and acclimated microorganisms to the different industrial wastewaters and fluctuations in their quality and quantity make their handling difficult (Ganezareyk, 1983).

Different solutions have been offered to deal with the start-up, operation and control problems of the biological wastewater treatment systems so far. A recently developed method is to prepare and preserve dried concentrated sludge from the sludge of the existing systems that function well for the removal of target pollutants. Therefore,prolonged preservation conditions of the sludge to be used later should be kept so that microorganisms can sustain their viability (McGhee and McCarty, 1990; Jones, 1998; Tchobanoglus et al., 2003). Various methods have been developed to store microorganism including bacteria. One of the best methods to preserve and store prepared sludge with keeping the microorganisms viability is lyophilization that can be used for large amount of sludge and for industrial scale.

Lyophilization is the creation of a stable preparation of a biologic substance by rapid freezing and dehydration of the frozen product under high vacuum (WHO, 1991; Kurosawa et al., 1997).

The main advantages of lyophilization are:

1. Keeping the bacterial quality of sludge for a long time;
2. Easy and inexpensive transportation and preservation of the prepared sludge;
3. Reduction of the sludge weight due to its dryness;
4. Availability of the prepared sludge to be used when needed;
5. Rapid start-up of the collapsed biological systems;
6. Pollution control due to wet sludge handling;
7. Environmental pollution control because treatment systems stabilized rapidly;
8. Possible use of the prepared sludge by this method for point-source pollution control;
9. Ease of the treatment process control regarding MLVSS, etc; and
10. Profit-making through the lyophilization sludge marketing in industrial scale.

This study was carried out to investigate the effect of lyophilization on the microbial quality of the activated sludge. In this regard, four operational parameters of the activated sludge including Oxygen Uptake Rate (OUR), Sludge Volume Index (SVI), soluble Chemical Oxygen Demand (COD) removal efficiency and predominant microorganisms' species before and after sludge lyophilization were studied in a lab-scale experiment.

MATERIALS AND METHODS

During this study, a bench scale activated sludge reactor with return settled sludge was developed. A schematic diagram of the investigated reactor is shown in Fig. 1.The reactor was consisted of two parts: aeration tank with 10 L in working volume and 12 h in Hydraulic Retention Time (HRT) and settling tank with 2.8 L in working volume and 3.4 h in HRT. Aeration tank was equipped with a mixer and a fine bubble aerator.

Experimental treatment operation consisted of two distinct steps: before lyophilization and after lyophilization synthetic wastewater composition used in both studies was the same, having organic load of 400 mg/L as COD in addition to all of microbial nutritional requirement and glucose was used as a sole carbon source. At the beginning of the first step of the study, reactor was seeded with activated sludge from a local municipal wastewater treatment plant. The initial MLSS concentration in the aeration tank was 1.2-1.3 g/L After seeding, effluent quality of the reactor was monitored until reaching steady state.

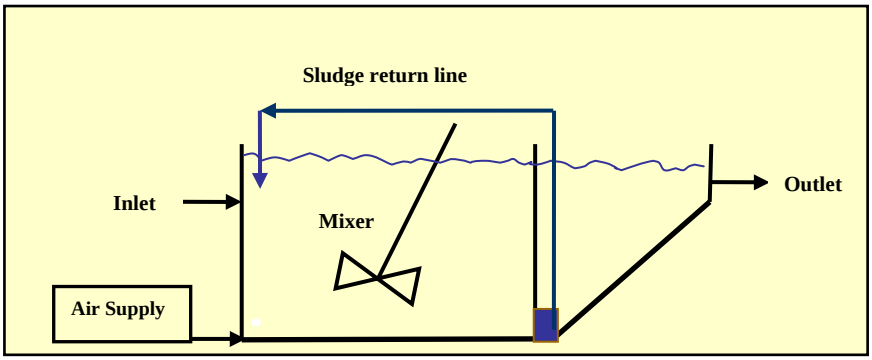

Fig. 1: Schematic of the experimental bench scale reactor

For investigation of the effect of lyophilization on selected activated sludge operation, parameters and characteristics including COD removal efficiency, SVI, OUR and microscopic examinations (to identify species of predominant organisms), this reactor was operated in 5 different Mean Cell Residence Times (MCRT) of 3, 5, 9, 13 and 16 d; in each of runs all above mentioned parameters were determined in steady state conditions before and after sludge lyophilization. Synthetic wastewater was pumped to the reactor at a flow rate of 20 L/d through a dosing pump during both step of the study. Species of predominant organisms were determined according to color atlas (APHA, 1998). The results obtained from two steps of the study were analyzed using SPSS software. All of examined parameters were carried out according to standard methods (APHA, 1998).

Lyophilization of activated sludge

For reactor start up in the second part of the study, lyophilization sludge prepared from mixed liquor of first step was used as sole source of seeding with influent wastewater which was in the same composition with that of before lyophilization. Sludge form run 2 in the first step of the study was selected for lyophilization. Cells were harvested by centrifugation of mixed liquor at 5000 rpm for 15 min and washed twice with cold distilled water. Cells were stored at 4 ºC until use. The centrifugully concentrated sludge was added in to special vials. The vials containing cells were frozen by dipping in an ethanol bath with temperature of-25 ºC, and immediately were connected to a lyophilizer apparatus with 60 Pa in pressure. Drying was performed under vacuum condition for 4-5 h. lyophilized sludge was stored at dark and room temperature for 3 months before adding to the reactor as a seed. To assay the effect of lyophilization and storage on viability and ability of microorganisms to remove organics, lyophilized cells were rehydrated with distilled water to give a cell suspension before adding to reactor as seed in the second part of the investigation.

Composition of reaction mixture in the reactor and also all of the operational conditions and experiments during this step was the same as before lyophilization.

Reactivation of lyophilized microorganisms

Stored lyophilized cells were rehydrated with distilled water and added to the reactor having all of nutritional requirements.

Reactor was monitored for a week in batch mode to increase biomass. During this period, mixed liquor was aerated and mixed. After reaching MLSS to 1200 mg/L, the reactor was operated in continuous mode and synthetic wastewater was pumped in to the reactor at a flow rate of 20 L/d.

RESULTS

As mentioned earlier, at the beginning of the first step of this study, reactor was filled approximately with 2 litters of activated sludge from municipal wastewater treatment plant. During starting-up, MLSS of the seeded

wastewater in the aeration tank was maintained approximately at 1200 ± 50 mg/L. The seeded wastewater in the aeration tank was then aerated at a constant air flow rate. Synthetic wastewater was pumped to the reactor at a flow rate of 20 L/d through a dosing pump. Start up of the reactor was released at the temperature of 23 ± 2 ºC and a constant HRT of 12 h. After reaching to the steady state in the effluent quality of the reactor in the first step of this study, operational parameters of COD, MLSS, MLVSS, OUR, SVI and species of predominant organisms were determined.

The mean values of influent and effluent COD and those for aeration tank MLSS, MLVSS, OUR, SVI and species of predominant organisms in the different runs are given in Table 1.

Table 1: Mean values of tested parameters in each of 5 runs before lyophilization

Run	$SCOD_{in}$* (mg/L)	$SCOD_{eff}$ ** (mg/L)	MCRT (day)	MLSS (mg/L)	MLVSS (mg/L)
1	400	31.5	3.2	1213	945
2	400	27	4.9	1664	1298
3	400	22.5	8.9	2480	1948
4	400	19.5	13	2970	2317
5	400	18	16.1	3665	2896

*Soluble chemical oxygen demand in influent

** Soluble chemical oxygen demand in effluent

When experiments of the first step were completed, the reactor was discharged, washed and prepared for the second part of investigation. Table 2 indicates the average values of operational parameters of the tested reactor after adding lyophilized sludge.

Table 2: Mean value of tested parameters in each of 5 runs after lyophilization

Run	$SCOD_{in}$ (mg/l)	$SCOD_{eff}$ (mg/l)	MCRT (day)	MLSS (mg/l)	MLVSS (mg/l)
1	400	25	3.4	1114	869
2	400	29	4.5	1433	1118
3	400	24	9.1	1970	1556
4	400	22	13.3	2774	2136
5	400	21	16	3020	2356

Figures 2 and 3 show the values and profile of SVI and OUR in any run before and after lyophilization, respectively.

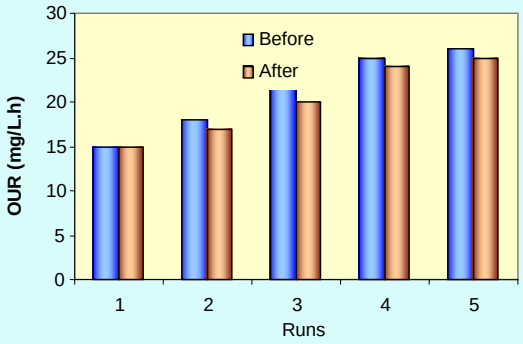

Fig. 3: OUR profile before and after lyophilization in different runs

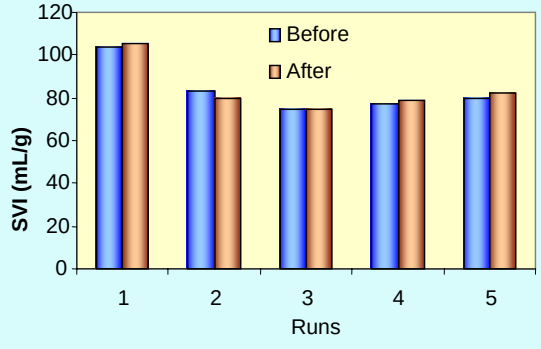

Fig. 2: SVI profile before and after lyophilization in different runs

Species of predominant organisms during each of two steps of the study are given in Table 3.

Table 3: Predominant of organisms' in different runs before and after lyophilization

Step	1	2	3	4	5
Before lyophilization	*Paramecium chilodonella*	*Paraeccium opercularia*	*Vorticella epistylis*	*Epistylis Philodina*	*Epistylis Philodina*
After lyophilization	*Paramecium chilodonella*	*Paraeccium opercularia*	*Opercularia Vorticella*	*Vorticella Philodina*	*Epistylis Philodina*

Discussion

In the present investigation, effect oflyophilization was studied using a bench scale activated sludge reactor. To assay the effect of lyophilization and study on viability and performance of cells, results from before and after sludge lyophilization were compared statically using SPSS software. It should be emphasized that, we could not find any research related to our study in literature.

Comparison of COD removal efficiency

The ability of the lyophilized activated sludge to remove organic matter was examined. The mean values of COD removal before and after lyophilization were 94% and 93% respectively. Statistical analysis indicated that with the confidence interval of 95%, efficiency of the reactor in COD removal had not any significant difference. So, after added to the suitable medium, cells have been able to reactivate and metabolize organics. It showed that the COD removal had not been affected so strongly by lyophilization of cells.

Comparison of SVI and OUR

As indicated in Tables 1 and 2, average SVI and OUR for tested reactor in both of two steps of the study had approximately same trends. According to the statistical analysis with a confidence interval of 95%, mean values of SVI and OUR before and after lyophilization had not any significant difference. This shows that the lyophilization has not had adverse affect on floc-forming cells and methabolitical ability of them, So that when were added to supporting medium, lyophilized cells would be able to reactivate.

Predominant organisms

Species of predominant organisms during both of investigation steps are given in Table 3. As it is observed, before lyophilization in run 1, free-living ciliates such as *Paremecium* were predominant. In run 2, attached ciliates were found and during run 3 (MCRT of 9 days) they were completely predominated. Population of *Rotifers* was increased gradually during run 4 and 5. After lyophilization, predominant organisms had the same variation trend as it was before sludge lyophilization (step 1).

In summary, any variations in operational parameters and activated sludge characteristics were not statistically significant during the two steps of this investigation.

Therefore, it was determined that the lyophilized cells were able to retained sufficient activity for removal of organics and to preserve their viability after lyophilization in the presence requirement. So, lyophilization was concluded to be a suitable technology for preparation and preservation of cells in activated sludge because of preserving viability, ease of handling and simplicity.

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