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Enhancement of bioseparation and dewaterability of domestic wastewater sludge by fungal treated dewatered sludge

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Abstract

A promising biological, sustainable, non-hazardous, safe and environmental friendly management and disposal technique of domestic wastewater sludge is global expectation. Fungal entrapped biosolids as a result of prior fungal treated raw wastewater sludge was recycled to evaluate its performance as inoculum for bioseparation/bioconversion of supplemented sludge in view of continuous as well as scale up wastewater sludge treatment. Encouraging results were achieved in bioseparation of suspended solids and in dewaterability/filterability of treated domestic wastewater sludge. Fungal entrapped biosolids offered 98% removal of total suspended solids (TSS) in supplemented sludge treatment at 6-day without nutrient (wheat flour, WF) supply. Consequently, 99% removal of turbidity and 87% removal of chemical oxygen demand (COD) were achieved in supernatant of treated sludge. The lowest value (1.75×10^{12} m/kg) of specific resistance to filtration (SRF) was observed at 6-day after treatment, which was equivalent to the 70% decrease of SRF. The all results except SRF were not influenced further in treatments accompanied with WF supplementation. The present treatments offered significant ($P \leq 0.01$) improvement in all results except SRF of treated wastewater sludge compared to the control. Furthermore, the present result is addressing a potential avenue of probable solution for expected management and disposal of domestic wastewater sludge in future.

Keywords: Bioseparation; Dewaterability; Fungal entrapped biosoilds; Domestic wastewater sludge

1. Introduction

Characteristically the activated sewage treatment plant sludge contains huge amount of water (approximately 96–99% water) along with organic solids [1–3]. This huge amount of water causes severe problem of its transportation as well as disposal. Since, separation of suspended solids and dewatering are considered as important factor for its proper management and disposal in treatment plants. The efficiency of solids–liquid separations successfully depends on the physical characteristics of the sludge particle aggregates such as size, density, porosity as well as the settling velocities. The poor settlement of activated sludge flocs adversely affects the operation of sewage treatment plants. There are several reports on using polymer, chemicals, polyelectrolyte, membrane filtration and flocculation for enhancement of settleability and dewaterability of sludge [4–7]. Mostly these advanced techniques are effective for non-soluble substances but inefficient for soluble and toxic pollutants. In addition, these are quite expensive and incapable of providing environmentally safe and friendly disposal [8]. On the other hand, biological-based treatment or bioremediation is a natural process in which microbial communities play important roles in break down or alteration the structure of sludge constituents [9–13].

However, in liquid state bioconversion, sludge particles were entrapped by filamentous mycelia that modify the porosity structure of the treated sludge and enhance separation and bioremediation significantly [14–16]. Fungal treatment tremendously improves dewaterability and filterability of treated wastewater sludge as compared to untreated sludge [15–17]. Moreover, a significant improvement in bioseparation and dewaterability of raw domestic wastewater sludge was observed in our another recent research after filamentous fungal treatment

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[unpublished]. Here it is also mentionable that our previous all researches on biological treatment of wastewater sludge were concerned about sterilized sludge. No doubt pre-sterilization practice has some impact to change the originality of the sludge structure. In contrast, the fungal inoculum is the key component and plays important roles for successful bioseparation of solids and bioremediation of wastewater sludge treatment. For continuous treatment it is important to ensure the continuous supply of potential fungal inocula. Definitely in higher scale of wastewater sludge treatment its requirement is also higher. No doubt its preparation and maintenance to its purity is laborious. Therefore, considering this point of continuous and scale up wastewater sludge treatment, the present research was assigned to evaluate the performance of fungal treated dewatered sludge (fungal entrapped biosolids after dewatering) as inocula in bioseparation and dewaterability of supplemented raw wastewater sludge treatment.

2. Experimental

2.1. Sample collection

For the present study the domestic wastewater sludge was collected from an aeration tank of Indah Water Konsortium (IWK) Treatment Plant, Kuala Lumpur, Malaysia and preserved it in plastic container in colds room at 4 °C for next immediate use. Thereafter, the total solids (%), total suspended solids (TSS) (%), turbidity (NTU), chemical oxygen demand (COD) (mg/L) and pH of the fresh sample (raw sludge) were measured as 1.21, 1.06, 4485, 3065 and 6.76, respectively, just after collect.

2.2. Preparation of fungal spore suspension

The fungus *Mucor hiemalis* Wehmer, isolated from IWK wastewater sludge cake in our previous studies [18] was used in the present research project. The fungal strain was subcultured on potato dextrose agar (PDA, 3.9% Merck) plate for 7 days at ambient temperature (28 ± 2 °C). For preparing spore suspension each plate of fungal culture was washed with 10 ml of 0.01% Tween 20 sterilized solution. Afterward the mycelial suspension was filtered by 12.5 cm Whatman #1 filter paper. The viable spores strength in fungal spore suspension was measured 3.03×10^6 (spores/ml) by serial dilution and plate count method. For counting viable spores, PDA media were used for germination of spores at ambient temperature (28 ± 2 °C) for 2–3 days. The stock of spores suspension was preserved at 4 °C and same source of fungal spores suspension was used for the present study.

2.3. Inoculum (fungal treated dewatered sludge/biosolids) preparation

The dewatered biosolids (sludge with fungal biomass, Fig. 1B) were obtained after dewatering of supernatant of initial fungal treated raw wastewater sludge (Fig. 1A). Successively, the dewatered biosolids were recycled as inoculum for further raw wastewater sludge treatment in the present study. However, the dewatered biosolids were prepared in following steps. Two percent fungal spores suspension (3.03×10^6 spores/ml) was added to 2% WF solution (prior sterilized at 121 °C for 15 min) for fungal broth preparation. The fungal broth was cultured in 250 ml Erlenmeyer wide mouth flask at 150 rpm in an orbital shaker for 60 h at 32 °C. Prepared 50 ml fungal broth was used for 100 ml raw wastewater sludge treatment was operated in single 250 ml Erlenmeyer flask at 150 rpm in an orbital shaker at 32 °C. After 3 days, the fungal biomass (broth) successfully separated all suspended solids. Discarded the clean supernatant (free water by pouring) and the whole dewatered biosolids (i.e. the content of each flask, Fig. 1B) were used for subsequent raw wastewater sludge (for 50 or 100 ml sludge based on experimental design) treatment for the present study.

2.4. Experimental design and statistical analysis

The present experiment was conducted in completely randomized design (CRD) with following five treatments and replicated five times. The assigned treatments were: (i) control (fresh 100 ml raw sludge only), (ii) 50 ml raw sludge without wheat flour (WF), i.e. 50 (−WF), (iii) 50 ml raw sludge with 1% WF, i.e. 50 (+WF), (iv) 100 ml raw sludge without WF, i.e. 100 (−WF), and (v) 100 ml raw sludge with 1% WF, i.e. 100 (+WF). In all situations except control, the almost same amount of fungal entrapped biosolids inocula (the used inocula were obtained from a single flask contained 50 ml fungal broth plus 100 ml sludge, explained in Section 2.3) were used for sludge treatment. For fungal treatment, flasks were operated at 150 rpm in an orbital shaker for 6 days at 32 °C. Parameters were studied in 48 h intervals since 0-day. The analysis of variance was accomplished in Microsoft Office Excel. The means were tested by least significance difference (LSD) at $P \leq 0.01$ level of significance.

2.5. Analytical methodology

The inoLab (Multi Level) pH meter was used to record the pH. Supernatant was used for analyzing of total suspended solids, turbidity and chemical oxygen demand. Ten millilitre supernatant was collected by pipette from upper surface of the treated sludge (from Fig. 1C) within 10–15 min after removal of flask from the shaker. After that 10 ml supernatant was diluted to 100 times with distilled H_2O for assessing the necessary parameters. The standard methods [19] were followed for TSS and COD measurement, while the Hach Turbidity meter 2100N was used for measurement of turbidity of the supernatant. Specific resistance to filtration (SRF) measures the resistance of sludge to filtration or dewaterability was analyzed based on description of our previous report [20]. Fisherbrand (code FB59021) 9 cm filter paper was used to collect 40 ml filtrate as a function of time by applying vacuum pressure of 300 mmHg. The following formula was used for specific resistance to filtration (SRF = r) calculation.

$$r = \left[\frac{2A2P}{\mu c^*}\right] b$$

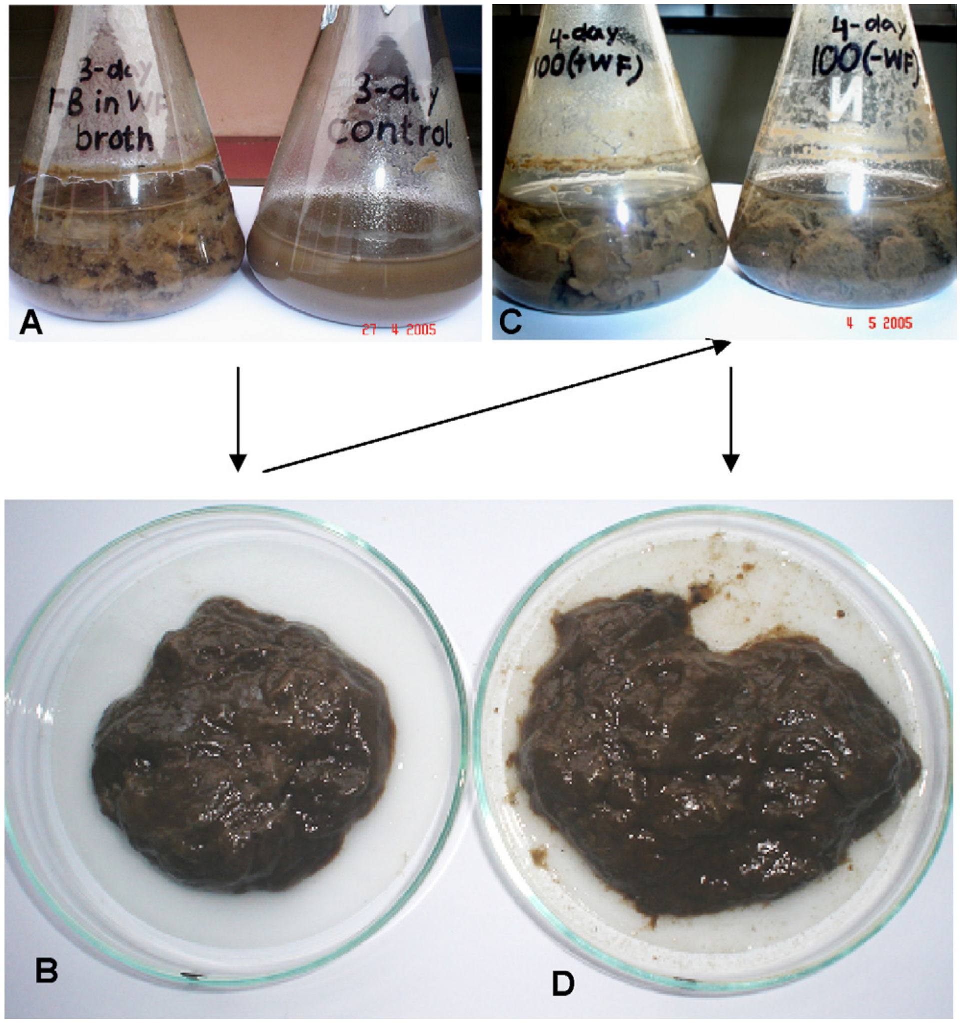

Fig. 1. Bioseparation of suspended solids and dewatering of supplemented raw domestic wastewater treatment plant sludge by filamentous fungus (*Mucor hiemalis*) entrapped biosolids from prior treated wastewater sludge. A: Raw 100 ml wastewater sludge after 3 days fungal (*Mucor hiemalis*) treatment with previously grown fungal inocula (the used 50 ml inocula was prepared in 2% sterilized wheat flour solution with 2% spore suspension in 48 h culture in rotary shaker), B: Fungal entrapped biosolids after dewatering/decanting of supernatant of A, C: Raw 100 ml supplemented wastewater sludge after 4 days treatment with dewatered fungal entrapped biosolids B, D: Decanted or fungal entrapped biosolids after dewatering of C.

where r is the specific resistance to filtration (m/kg), A the area of the filter paper (m^2), P the pressure of filtration (N/m^2), μ the viscosity of filtrate ($N\,s/m^2$), c^* the weight of dry solids per volume of filtrate (kg/m^3), b the slope of the plot of filtration time/filtrate volume (t/V) versus filtrate volume (V).

3. Results and discussion

3.1. Total suspended solids

The total suspended solids of raw wastewater sludge were decreased significantly ($P \leq 0.01$) after treatment by fungal entrapped biosolids (Fig. 2). Among the treatments, 50 (+WF) and 100 (+WF), i.e. 50 ml raw sludge with 1% (v/w) wheat flour (WF) and 100 ml sludge with 1% WF exhibited higher values of TSS at 0-day compared to the others. Conversely at 0-day the TSS was minimum in control treatment implied that the added inocula and WF enhanced TSS initially. However, the obtained values of TSS in fungal treated sludge were more or less similar and significantly lower compared to the control at 2-day. At 6-day the recorded TSS values in supernatant of treated wastewater sludge were 4500, 500, 300, 200 and 200 mg/l, while these were 9300, 13,700, 26,800, 14,100 and 24,900 mg/l at 0-day in control, 50 (−WF), 50 (+WF), 100 (−WF) and 100 (+WF) treatments, respectively. The decreasing trend of TSS was maximum at higher volume of sludge (i.e. in 100 than 50 ml) but it was insignificant. Perhaps orbital shaking and volume of the sludge had some correlation on separation of suspended solids. On the other hand, the removal percent of TSS in treated sludge at 6-day was 51.6, 96.3, 98.9, 98.6 and 99.2 in control, 50 (−WF), 50 (+WF), 100 (−WF) and 100 (+WF) treatments, respectively. The almost maximum percent removal of TSS was attained at 2-day, which was above 85% in WF added treatments. Relatively higher percent removal of TSS was occurred in WF added treatments but which was insignificant with non-added treatments (Fig. 2). Entrapping of suspended solids by fungal filamentous structure is the probable mechanism of decreasing TSS

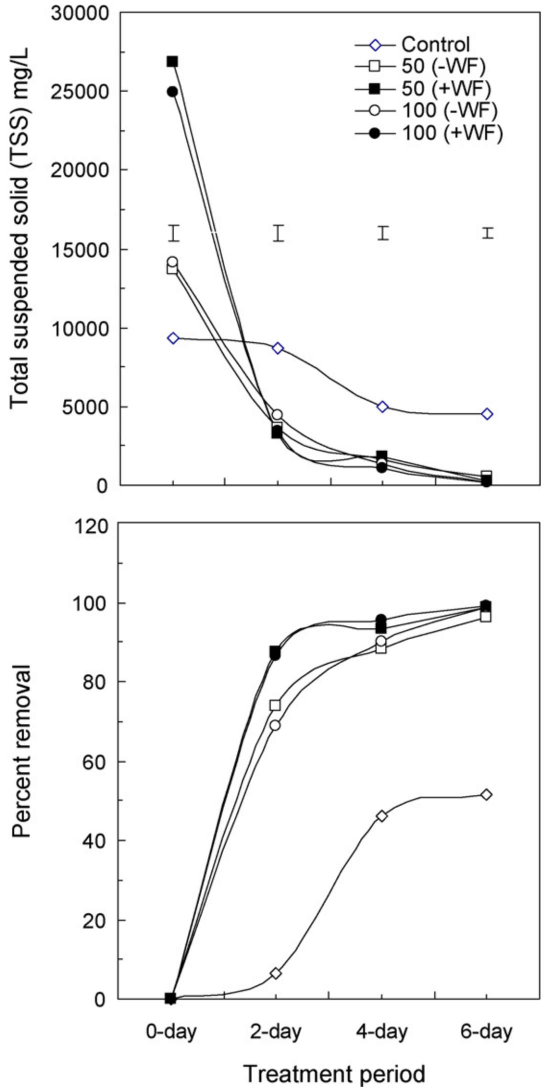

Fig. 2. Total suspended solids (TSS) in supernatant of treated supplemented raw domestic wastewater sludge by fungal entrapped biosolids. Vertical bars represent the LSD at $P \leq 0.01$ level.

in treated wastewater sludge. Similarly, significant entrapping of suspended solids in supplemented raw sludge was observed by fungal entrapped biosolids in present studies (Fig. 1BCD). Moreover, there are several citations in literature on decreasing trends of TSS by fungal treatments of several wastewater sludge. Alam et al. [20] recorded the minimum value of TSS 93 mg/l and maximum removal of TSS 98.8% after 8 days of fungal treatment of pre-sterilized domestic wastewater sludge. They reported the higher reduction rate was attained after 6 days and no remarkable changes were noticed hereafter. Entrap of suspended solids particles in sludge by filamentous fungi results enhanced significant reduction of TSS [15,21].

3.2. Turbidity

The trend of turbidity in supernatant of treated raw wastewater sludge by fungal entrapped biosolids was followed almost similar profiles as TSS. At 2-day the turbidity decreased significantly ($P \leq 0.01$) in all fungal treatments compared to the control (Fig. 3). The obtained results showed that the recorded values of turbidity were 4510, 6086, 11,000, 7095 and 10,553 (NTU) at 0-day, which were decreased to 2565, 129, 44, 40 and 36 (NTU) at 6-day in control, 50 (−WF), 50 (+WF), 100 (−WF) and 100 (+WF) treatments, respectively. At 2-day period of treatment the removal percent of turbidity was attained 5.1, 93.3, 94.9, 92.3 and 93.6 in control, 50 (−WF), 50 (+WF), 100 (−WF) and 100 (+WF) treatments, respectively, and simultaneously these values were recorded 43.1, 97.9, 99.6, 99.4, and 99.6% in control, 50 (−WF), 50 (+WF), 100 (−WF) and 100 (+WF) treatments, respectively, at 6-day period. The obtained results of turbidity implied that the previously fungal treated sludge residues, i.e. fungal entrapped biosolids were quite efficient to remove almost all suspended wastes/solids from supplemented raw wastewater sludge. The effect of nutrients (WF) supply on enhancement of removal turbidity was insignificant compared to the non-added WF treatment (except control). Assurance of nutrients supply for proper fungal growth was the reason for WF application in the system. Perhaps the sludge itself accomplished this pur-

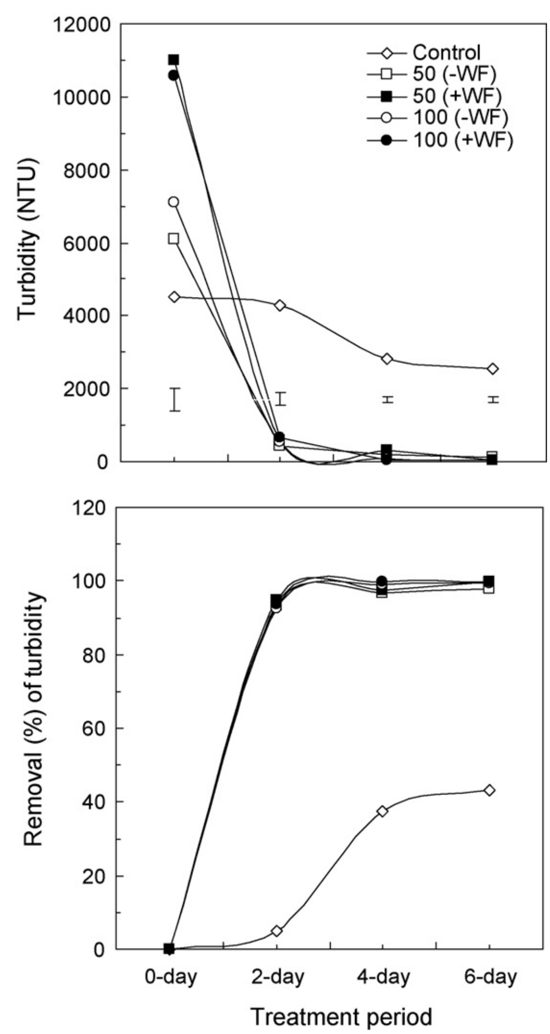

Fig. 3. Turbidity profiles in supernatant of treated supplemented domestic wastewater sludge by fungal entrapped biosolids. Vertical bars represent the LSD at $P \leq 0.01$ level.

pose. Therefore, the WF added treatment did not offer significant results. Decreasing trends of turbidity in wastewater sludge by fungal treatment were reported by several authors but all of these reports described the use of sole fungal inocula. Accordingly, at 2-day 99% removal of turbidity in filtrate (not in supernatant) of activated sludge treated by *Penicillum corylophilum* was reported by Mannan et al. [22]. Moreover, maximum 97.3% turbidity removal was recorded at 8 days after treatment of domestic wastewater sludge by filamentous fungus [20]. In another report, significant decreasing of turbidity in wastewater sludge after fungal treatment was also reported by Alam and Fakhru'l-Razi [12].

3.3. Chemical oxygen demand

Chemical oxygen demand of the supernatant of treated sludge was decreased significantly ($P \leq 0.01$) by fungal entrapped biosolids (Fig. 4). The gradual decreasing trends of COD were observed in all treatments. But the highest decreasing was noticed in 100 (−WF) and the lowest was in control treatment. At 6-day after treatment of wastewater sludge by fungal entrapped biosolids the recorded values of COD in supernatant were 2750, 480, 880, 400 and 480 mg/l, whereas these values were 3000, 3200, 3680, 3120 and 3600 mg/l at 0-day in control, 50 (−WF), 50 (+WF), 100 (−WF) and 100 (+WF) treatments, respectively. Conversely, the percent removal of COD at 6-day was 8, 85, 76, 87 and 86 in control, 50 (−WF), 50 (+WF), 100 (−WF) and 100 (+WF) treatments, respectively. Besides, the decreasing rate of COD was relatively higher in non-added wheat flour (−WF) treatment compared to the added one but which was insignificant to each other. However, in our earlier report around 82% removal of COD was monitored in sterilized domestic wastewater sludge by the treatment of fresh fungal inocula [20]. Definitely the percent removal of COD depends on the effective performance of microbes such as break down or alteration in the structure of sludge constituents [9,10,12] as well as the amount of organic substances presence in the system. Significant percent of COD removal in the filtrate of treated activated domestic wastewater sludge by filamentous fungi was also reported by Mannan et al. [22]. Moreover, the biological-based treatments also reported the COD removal of pharmaceutical wastewater [23], olive mill wastewater [9], saline wastewater [24] and textile wastewater [25].

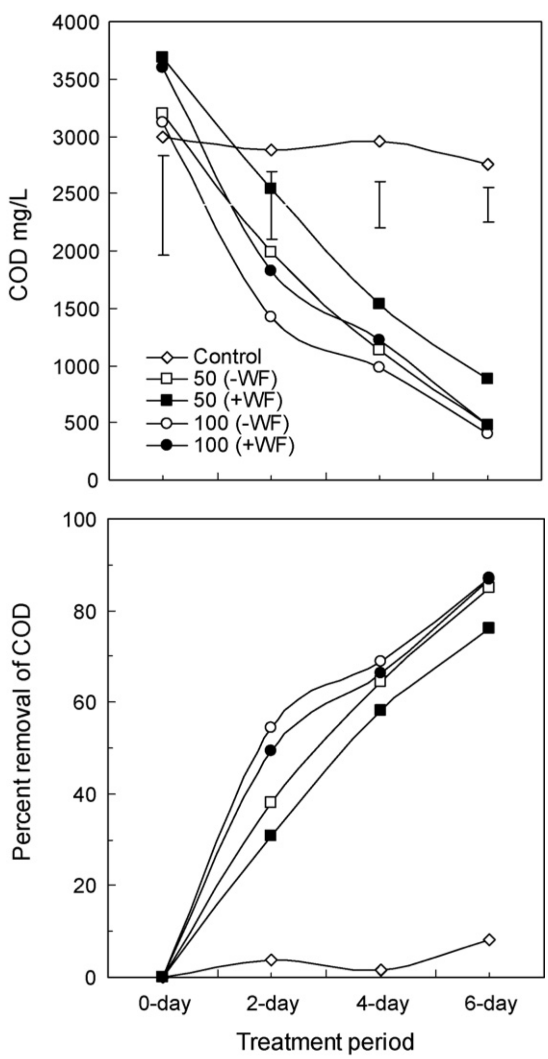

Fig. 4. Chemical oxygen demand (COD) in supernatant of treated supplemented domestic wastewater sludge by fungal entrapped biosolids. Vertical bars represent the LSD at $P \leq 0.01$ level.

3.4. Specific resistance to filtration

Specific resistance to filtration was monitored to assess the dewaterability/filterability of treated sludge. The dewaterability of supplemented raw domestic wastewater sludge was influenced over time after treatment by filamentous fungal entrapped biosolids (Fig. 5). The values of SRF (m/kg) were decreased in all treatments. The changing profiles of two wheat flour (WF) added treatments followed similar trends but it was different with non-added (−WF) treatments. The SRF values at 2-day were higher at both WF added treatments. In practical observation in the present study suggested that the WF using substrate was changed into too gluey (not measured) at 2-day, therefore it imposed resistance to filtration and finally it enhanced in higher SRF record. But at following date of sampling (at 4-day) it almost disappeared. The gluey characteristics were also remarked at non-added WF treatments but it was minimum at 2-day than that of WF added treatments. Also at non-added WF treatment it disappeared slowly than the WF added treatments. However, the gluey/gooey characteristics were absent in control flasks. Besides both fungal entrapped biosolids and quantity of sludge had prominent roles in filtration of treated sludge. The entrapped biosolids mass (mud like structure, Fig. 1B) made resistance in filtration of treated sludge compared to the control. Conversely the water content at lower amount of sludge (50 ml) was mostly absorbed with fungal entrapped biosolids than the higher amount of sludge (100 ml) as well. That situation also enhanced resistance in filtration at treatments of 50 ml sludge than the 100 ml. Therefore, the SRF values were the lowest in control. Addition of WF enhanced resistance, i.e. increased SRF values compared to others at early periods of treatment. However, at 6-day the difference of SFR reading of control with 100 (−WF) and 100 (+WF) treatments was minimized and finally the WF added treatments offered superior performance in filtration of treated sludge compared to the non-added (−WF) sludge. The obtained results (Fig. 5) showed that the maximum 70.3% decrease of SRF was achieved in the treatment of 100 (+WF) and the minimum 24.6% was observed in 50 (−WF) treatment at 6-day. About 90% decrease of SRF at 2 days was reported in treated activated wastewater sludge by fresh filamentous fungi treatment [22]. Conversely 98% decrease of SRF was reported at 6 days in treated sludge also after treatment by fresh fungal broth [20]. In the present study, comparatively poor results of SRF decrease (%) were achieved compared to the previous reports. But the tendency of the trends (Fig. 5) showed that the SRF might decrease in some extent at fungal treatments if the treatment period to be extended. Using of fungal entrapped biosolids and lower amount (50 ml) sludge offered the poor and delayed results of SRF in the present study. It might be the reasons of inferior results than the previous reports.

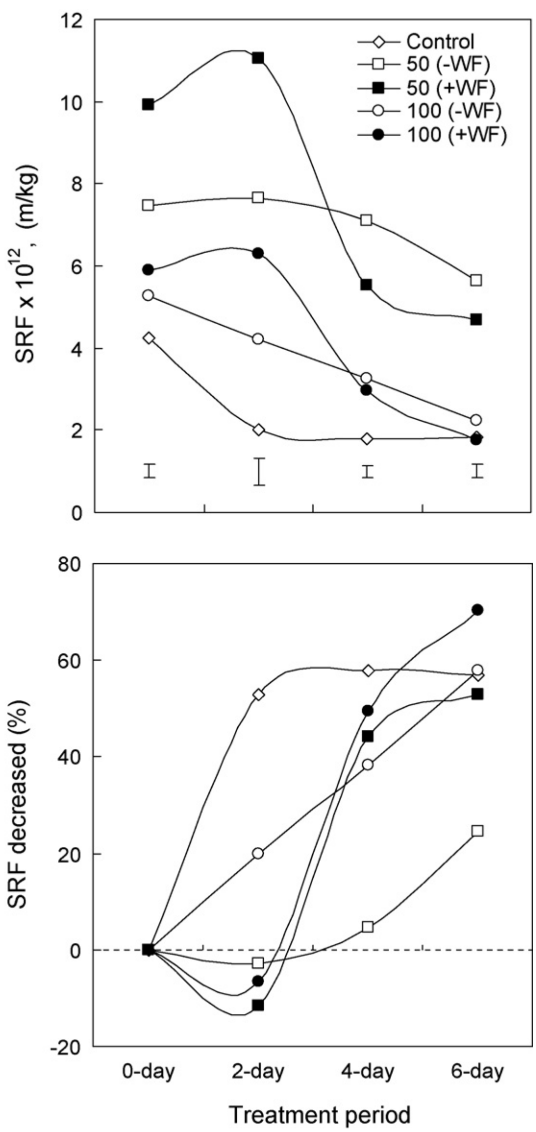

Fig. 5. Changes pattern of specific resistance to filtration (SRF) of treated supplemented domestic wastewater sludge by fungal entrapped. Vertical bars represent the LSD at $P \leq 0.05$ level.

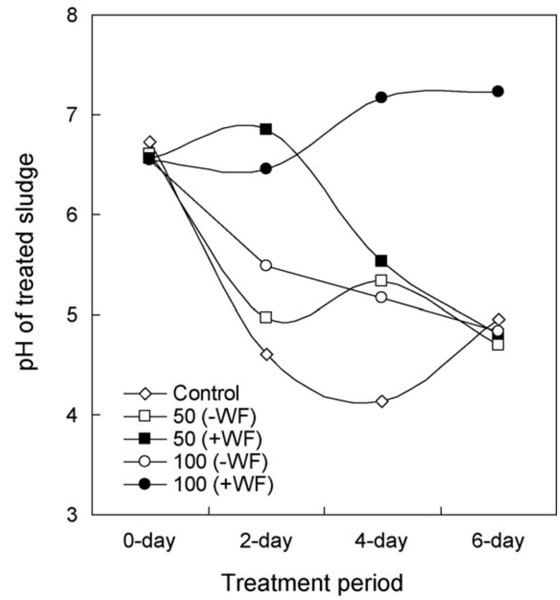

Fig. 6. The profiles of pH of treated supplemented domestic wastewater sludge by fungal entrapped fungal biomass as inocula.

3.5. pH

Like other parameters, the pH decreased in treated supplemented raw wastewater sludge by fungal entrapped biosolids in all treatments except 100 (+WF). The recorded values of pH were 6.72, 6.61, 6.65, 6.55 and 6.55 at 0-day, which was changed into 5.12, 4.70, 4.81, 4.83 and 7.22, respectively at 6-day in treatments control, 50 (−WF), 50 (+WF), 100 (−WF) and 100 (+WF). The pH decreased profile was also remarked in control except at 6-day (Fig. 6). In control the pH profile was 6.72, 4.61, 4.14 and 5.12 at 0, 2, 4 and 6-day, respectively. Both WF added treatments projected increased values of pH but finally the treatment 50 (+WF) offered declined pH trend over time of treatment. On the other hand, the decreasing trends of pH were observed in sludge treated without WF application. The decreased pH values implied the intensity of fungal growth and multiplication generally in stress environment. Perhaps, the without added WF treatments enhanced it. Conversely, WF added treatment did not face this situation but the treatment 50 (+WF) might face this situation at later period of treatment due to scarcity of nutrient. In the present treatment 1% WF was supplied based on amount of supplemented sludge but the fungal inocula size was same in every treatment. The decrease of pH by microbial treatment of wastewater sludge was reported earlier by several authors [14,15,22].

4. Conclusions

The following conclusions were drawn based on above results and discussion:

(1) Fungal entrapped biosolids played significant roles in bioseparation of suspended solids and dewaterability/filterability of supplemented raw wastewater sludge.

(2) In supplemented sludge treatment by fungal entrapped biosolids, encouraging results were obtained in all treatments but the treatment 100 (−WF) was most suitable in all respect.
(3) The obtained results of the present study implied that the fungal entrapped biosolids from prior wastewater treatment would be capable of continuously treating supplemented raw wastewater sludge without nutrient supply to the system.
(4) Safe environmental management, dewatering and disposal of wastewater sludge is a common problem of each treatment plant. The present results are conveying the positive indication of continuous bioseparation of suspended solids in raw wastewater sludge. Perhaps it would able to contribute a significant improvement and address a potential avenue for environmental friendly management and disposal of wastewater sludge in future program.

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