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Evaluation of fungal potentiality for bioconversion of domestic wastewater sludge

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Abstract: This study was undertaken to screen the filamentous fungi isolated from its relevant habitats (wastewater, sewage sludge and sludge cake) for the bioconversion of domestic wastewater sludge. A total of 35 fungal strains were tested against wastewater sludge (total suspended solids, TSS 1%—5% w/w) to evaluate its potentiality for enhancing the biodegradability and dewaterability using liquid state bioconversion (LSB) process. The strains were divided into five groups i.e. *Penicillium*, *Aspergillus*, *Trichoderma*, Basidiomycete and Miscellaneous, respectively. The strains WWZP1003, SCahmA103, SCahmT105 and PC-9 among their respective groups of *Penicillium*, *Aspergillus*, *Trichoderma* and Basidiomycete played potential roles in terms of separation (formation of pellets/flocs/filaments), biodegradation (removal of COD) and filtration (filterability) of treated domestic wastewater sludge. The Miscellaneous group was not considered due to its unsatisfactory results as compared to the other groups. The pH value was also influenced by the microbial treatment during fermentation process. The filterability of treated sludge was improved by fungal treatment, and lowest filtration time was recorded for the strain WWZP1003 and SCahmA103 of *Penicillium* and *Aspergillus* groups respectively compared with other strains.

Keywords: filamentous fungi; screening; wastewater sludge; pellets; filtration; liquid state bioconversion

Introduction

Wastewater typically contains a complex mixture of components which are degraded by a diverse range of microbial cells in biochemical reactions. As biological, biochemical and physical phenomena all influence the nutrient removal, these will be considered in conjunction with strategies for process operation to identify the mechanism which may reduce the disposal requirements. Biological wastewater treatment involves the transformation of dissolved and suspended organic contaminants to sludge and evolved gases (CO_2, CH_4, N_2 and SO_2) which are separable from treated water (Low, 1999). In Malaysia, Indah Water Konsortium (IWK) organization of wastewater treatment and management faces a great problem in processing and disposing the enormous quantity of sludge generated from the treatment plants. The total cost of sludge management was estimated at USD 0.26 billion for the production of 3.2 million cubic meters of sewage sludge annually (Kadir, 1999).

From an operational and economical point of view, one of the most important steps in biological wastewater treatment is the separation and the removal of the excess sludge generated during the treatment process (WPCF, 1987). It is important to know the settling and dewatering characteristics of the sludge for proper operation of treatment process. The ultimate disposal of the sludge to the environment is usually required satisfactory solid handling operations such as thickening, stabilization, conditioning and dewatering. Sludge dewatering is the removal of water to change the sludge from semisolid state to damp solid. This physical change reduces the volume of the sludge considerably and also reduces the cost of disposal.

The separation and biodegradation of wastewater sludge can be achieved by fungal treatment using liquid state bioconversion process (Alam, 2002). In this process, microorganisms isolated from wastewater and sewage sludge (Fakhru'l-Razi, 2002a) will be immobilized in waste particles and as a result the sludge slurry will be clarified (Alam, 2002). Since the fungus entraps the solid particles of sludge, the settling and dewatering characteristics may be enhanced considerably (Alam, 2003). The soluble substances in wastewater sludge will be assimilated by the microorganisms during the bioconversion process. The recovery of coal fines by oil agglomeration technique and biofilm formation was successfully achieved by exploiting fungal activity (Sharma, 1999). The technique of biofilm formation is a low cost and environmentally friendly technique for the treatment of wastewater (Bryers, 1987; Tanaka, 1991). Therefore, the objective of this study was to evaluate the potential performance of filamentous fungi (isolated from relevant sources) based on their biodegradability and dewaterability of treated sludge by liquid state bioconversion process.

1 Materials and methods

1.1 Microorganisms

Thirty-five strains of filamentous fungi in five groups (*Penicillium*, *Aspergillus*, *Trichoderma*, Basidiomycete and Miscellaneous) were used in screening test against different solid content (%) of wastewater sludge (Table 1). All culture strains were maintained by subculturing on potato dextrose agar (PDA, Oxoid, England) medium slants once in a month and subsequently stored at 4℃.

1.2 Effluent source

The sample of domestic wastewater sludge ((Total suspended solids (TSS) 0.75% w/w, pH 6.6)) was collected from aeration tank of IWK's sewage treatment plant in Malaysia and stored in plastic container (30 L) at 4℃. The wastewater sludge (TSS 1%—5% w/w) medium containing 2% (w/w) of malt extract (ME) as co-substrate was used for fungal initial growth throughout this study.

1.3 Inoculum preparation

The inocula of spore suspensions were used for the sporulating strains of *Penicillum*, *Aspergillus*, *Trichoderma* and Miscellaneous, while mycelial suspension was used for basidiomycete group.

1.3.1 Spore suspension

The cultures were grown on PDA medium in petri dishes at room temperature(30 ± 2℃) for 7 d (*Trichoderma*, and Misc.) and 10 d (*Penicillium* and *Aspergillus*). Cultures of the plate were transferred into Erlenmeyer flask(250 ml) containing 100 ml of sterile distilled water. It was shaken in a rotary shaker with 150 r/min for 24 h. The suspended fungal cultures were filtered by Whatman # 1 filter paper. Finally the filtrate was used as inoculum after measuring its strength(spores/ml) by Haemocytometer. All flasks, funnel, filter paper, distilled water were sterilized prior to use.

Table 1 The strains of filamentous fungi from different sources for screening test

Group	Genus	Isolates			Lab stock				Total
		Wastewater	Sewage sludge	Leachate	POME	Oversease	Compost	Sludge cake	
Penicillium	*Penicillium*	WWZP1003	SSZP2015	LZP3001	PC-P502	0	0	0	11
		WWZP1005	SSZP2010	LZP3005	PC-P503				
		WWZP1008	SSZP2012						
		WWZP1009							
Aspergillus	*Aspergillus*	WWZA1006	SSZA2017	LZA3009	0	*Aspergillus niger*	0	SCahmA103	08
								SCahmA101	
								SCahmA109	
								SCA207	
Trichoderma	*Trichoderma*	0	SSZTz2008	0	0	0	CahmT409	SCahmT105	10
			SSZT2005					SCT202	
			SSZT1002					SCahmT108	
			SSZT2016					SCT201	
			SSZT2019						
Basideomycete	*P. chysosporium*	0	0	0	0	PC-9, PC-2094	0	0	2
	P. sajor caju	0	0	0	0	*P. sajor caju*		0	1
Misc.	*Spicaria*	WWZS1007	0	0	0	0	0	0	1
	Hyaloflorae	0	SSZH2006	0	0	0	0	0	1
	Myriodentium	0	0	0	0	0	0	SCM104	1
Total		6	10	3	2	4	1	9	35

1.3.2 Mycelial suspension

Seven days old culture grown on PDA plates were used for mycelial suspension. The mycelia in plates were washed successively three times with 50 ml of sterile distilled water by a glass rod and poured into 100 ml of glass tube for use as final inoculum.

1.4 Experimental procedures

The screening experiment was divided into two phases. Four treatments (control, 1% w/w, 2% w/w and 3% w/w of wastewater sludge) in the first phase and two treatments in the second phase (4% w/w and 5% w/w of wastewater sludge) were used to evaluate the fungal potentiality. The experiments were conducted in 250 ml Erlenmeyer flask containing 50 g of sludge samples incorporated with 2% of malt extract as co-substrate. The samples were autoclaved at 121℃ for 30—60 min and inoculated with 2% (v/w) of spores/mycelial suspension (10^3—10^6 spores/ml). Cultures were incubated in a rotary shaker with 150 r/min at 33 ± 1℃ for 3, 6 and 9 d. The initial pH of the samples were recorded (6.5—7.6) but not adjusted.

1.5 Analytical methods

The fungal growth was measured as dry weight of biomass. The culture grown in different TSS content of wastewater sludge was filtered and dried at 105℃ in an oven for 6—24 h. The fungal growth formation as pellets, flocs, filaments was observed visually and photographs were captured by an image analysis system consisting of a CCD camera, a PC and an image analysis software (Leica Qwin 5001). Total suspended solids(TSS) and COD were determined according to the standard method (APHA, 1989). The filterability of fermentation broth was determined by measuring the filtration time of every 5 ml of the filtrate. The filtration was carried out under constant vacuum of 600-mmHg (Friedrich, 1983). The results obtained were the average of three replicates.

2 Results and discussion

On the basis of the results of screening test against wastewater sludge, the experiments were carried out in two phases. A total of 35 strains of filamentous fungi were screened in first phase (Table 1) and those strains which could form pellets/flocs were selected for second phase experiment. The strains WWZP1003, SCahmA103, SCahmT105 and PC-9 among their respective groups of *Penicillium*, *Aspergillus*, *Trichoderma* and Basidiomycete (*Phanerochaete chrysosporium*) were performed to better potential by enhancing the biodegradability and dewaterability of sludge. The strains of SCahmT105 and PC-9 were chosen from the first phase experiment while strains WWZP1003 and SCahmA103 were selected by considering both phases. The fungi of Miscellaneous group were not considered due to the unsatisfactory results as compared to other groups. The various parameters were observed for the evaluation of strain performance in LSB process. The growth formation (filaments, flocs and pellets), dry cell biomass, increased TSS%, pH, and removal of COD (%) were measured in both phases but filterability test was observed only in second phase experiment.

2.1 Formation of pellets, flocs and filaments

The formation of pellets/flocs/filaments in lower solid content of DWTP sludge (TSS 1%—2% w/w) and entrapment of solid particles in higher solid content (TSS 3%—5% w/w) were observed visually (Figures are omitted). Pellets were formed by four strains of *Penicillium* (WWZP1003, LZP3001, LZP3005 and WWZP1008) group followed by 4 strains of *Aspergillus* (SCahmA103, SCahmA109, WWZA1006, and SSZA2017) and 2 strains of *P. chrysosporium* (PC-9, PC-2094) except *Trichoderma* (SCahmT105) which was formed into flocs/filaments. The strains of WWZP1003, SCahmA103, SCahmT105 and PC-9 among their groups were immobilized on solid particles of waste sludge by the growth formation of pellets/flocs and enhanced the separation and filtration process of treated sludge. Alam *et al*. (Alam, 2001; 2002) have studied that the fungal growth immobilized in solid particles of wastewater sludge which has been transformed into mycelial balls (pellets) and enhanced the separation process. In higher solid content of sludge, the solid particles of sludge were entrapped by the microbial growth and as a result they increased the dewaterability of treated sludge (Fakhru'l-Razi, 2002b; Hamdi, 1992). They were not able to form pellets/flocs in higher solid content of sludge due to the absence of free water. The formation of pellets/flocs may be influenced by the presence of solids content and free water in sludge which are good support for mycelial growth. It has been observed that suspended solids stimulated the germination of spores and enhanced the elongation and ramification of the mycelium (Hamdi, 1991).

2.2 Dry cell biomass

The dry cell biomass of filamentous fungi was estimated in wastewater sludge treatment (TSS 1%—5% w/w) to evaluate the bioconversion performance. The obtained results were compared to individual group not to other groups. The strains WWZP1003 (*Penicillium*), SCahmA103 (*Aspergillus*) and SCahmT105 (*Trichoderma*) produced highest biomass in the treatment of higher percentage of sludge (TSS 3%—5% w/w) after 9 d of treatment except for basidiomycete (PC-9) which produced highest biomass at 6 d (Fig. 1). The maximum biomass obtained in lower percentage of sludge (1%—2% w/w) was recorded up to 6 d treatment after that the growth was declined except for *Penicillium* where its maximum growth was recorded after 3 d of treatment (1% w/w). In all cases, the minimum biomass was measured in control treatment (2% of ME in distilled water) compared to other treatments. It might be due to the lack of sufficient nutrients, trace element in control for proper growth of microbes as compared to sludge containing media (Outwater, 1994). Individually *Penicillium* was the maximum biomass producer followed by *Aspergillus*, *Trichoderma* and *P. chrysosporium* respectively for 6 d (lower TSS) and 9 d (higher TSS) of treatments. The biomass production of *Penicillium* in olive mill wastewater isolated from its relevant sources has been studied by Robles *et al*. (Robles, 2000). Similar observations have been found by several authors with different microbes (Hamdi, 1993; Martirani, 1996; Setti, 1998; Vincigueria, 1995). Wastewater sludge contained organic substances, nutrients (N, P, K), trace elements that accelerate the proper growth of microorganisms. The growth of fungi was higher in higher percentage of solids that stored higher content of essential elements than the lower percentage of sludge, which could be the reason of their higher growth.

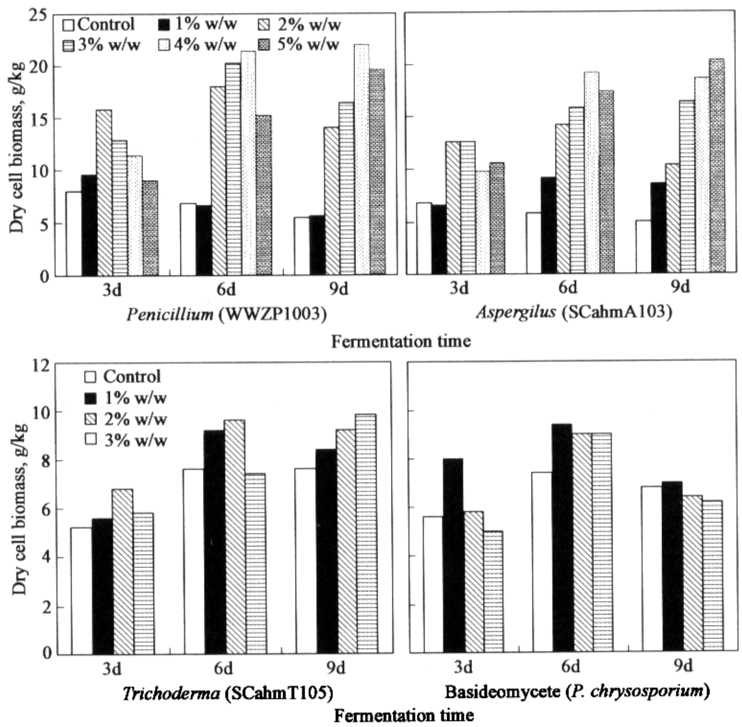

Fig. 1 Dry cell biomass was produced by the strains WWZP1003, SCahmA103, SCahmT105 and *P. chrysosporium*-9 of *Penicillium*, *Aspergillus* *Trichoderma* and Basidiomycete groups respectively

2.3 Increased percent of total suspended solids (dry sludge cake)

The effect of microbial treatment on increased percent of total suspended solids (TSS%) of sludge is shown in Fig.2. The TSS% (dry sludge cake) was measured with increased dry biomass of filamentous fungi and decreased total weight. The results showed that increased TSS% was higher in lower percentage of solids content (1%—2% w/w) than those of higher solid content (3%—5% w/w) of sludge. It occurred due to increase of the dry biomass of fungi and solid content of sludge. The highest increased TSS% was recorded for *Aspergillus* (SCahmA103, 114%) followed by *P. chrysosporium* (PC-9, 111%) and *Trichoderma* (SCahmT105, 106%) respectively in 1% w/w of sludge after 6 d except for *Penicillium* (WWZP1003, 104%), achieved it after 3 d of fermentation (Fig.2). The increasing trend was observed up to 9 d in higher solid content of sludge (2%—5% w/w) for all strains. *Penicillium* produced highest increased TSS% (58%—76%) in higher solid content of sludge compared to other groups (36%—70%) in treatment. The biodegradation of insoluble organic matters in sludge was lower than the biosynthesis of mycelium of filamentous fungi and enhanced the increased TSS%. The insoluble particle was hardly degraded by microbes and therefore the insoluble matter might increase on account of mycelial growth(Friedrich, 1983). Total weight loss and increased TSS% in 0.5 % w/w of wastewater sludge has been studied by Alam *et al*.(Alam, 2001) using *P. chrysosporium*. The results showed that increasing rate of TSS% was higher (164%) at 7 d than those of 14 (226%) and 21(276%) d.

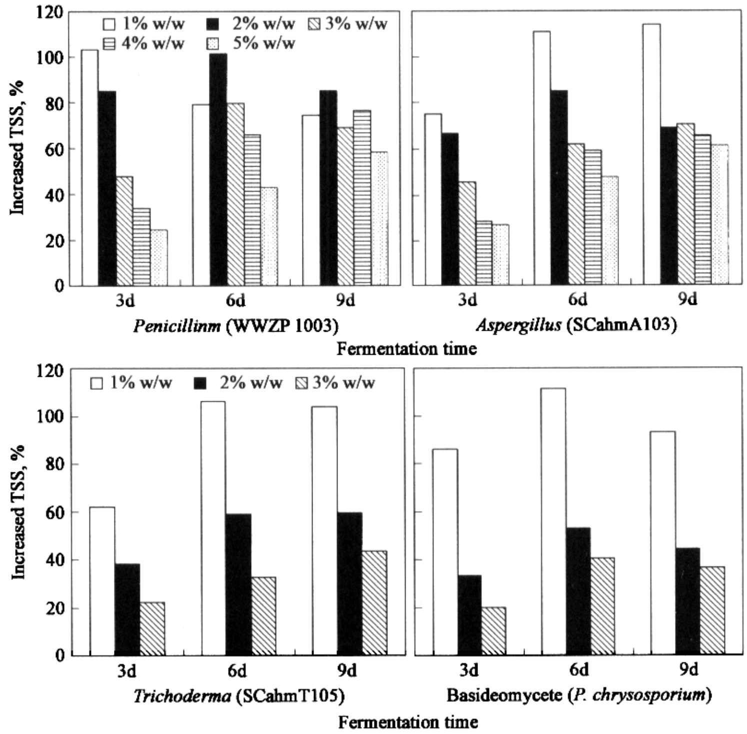

Fig. 2 The effect of fungal strains WWZP1003, SCahmA103, SCahmT105 and *P. chrysosporium*-9 of their respective groups of *Penicillium*, *Aspergillus*, *Trichoderma* and Basidiomycete on increased TSS% in wastewater sludge

2.4 Reduction of COD

The reduction of chemical oxygen demand(COD) in treated sludge after 9 d treatment is shown in Fig.3. The COD of the treated sludge was decreased by *Penicillium*, *Aspergillus*, *Trichoderma* and Basidiomycete groups to the extent of 29%—93%, 43%—86%, 40%—76%, and 45%—77%, respectively(data not shown). The maximum removal of COD recorded was 81%—93% by WWZP1003(*Penicillium*) followed by 66%—86% for SCahmA103(*Aspergillus*), 64%—77% for PC-9 (*P. chrysosporium*) and 45%—76% for SCahmT105(*Trichoderma*) respectively from their individual group for all treatments(Fig.3). The reduced COD values(66%—93%) by *Penicillium* and *Aspergillus* were higher than those of *Trichoderma* and *P. chrysosporium* (45%—77%) in treatments. In *Aspergillus*, the maximum COD removal was observed in strain SCahmA109 (77%) though the result was not significantly different as compared to strain SCahmA103 (76%). During the fermentation period the fungus utilized the soluble organic matters and as a result substrates were reduced and this process enhanced the COD reduction. The COD reduction was more pronounced in lower solid content of waste than that of higher solid contents(Friedrich, 1983). The reduction of COD has been studied by many authors in different waste treatment areas where the COD of treated domestic wastewater sludge was recorded by using *P. chrysosporium* (Alam, 2001; 2002). Similar results have been made by other authors using *Penicillium* (Robles, 2000), *Aspergillus* (Hamdi, 1991), *Lentinus edodes* (Vinciguerra, 1995), *Pleuroutus ostreatus* (Martirani, 1998) in olive mill waste (OMW); *Aspergillus* and *Trichoderma*(Friedrich, 1983; 1987) in apple distillery waste.

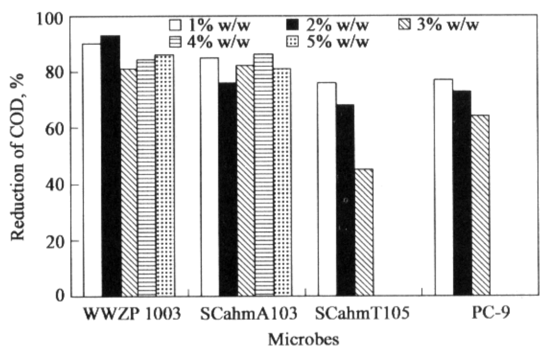

Fig. 3 Reduction of COD after 9 d of treatment using *Penicillium* (WWZP1003), *Aspergillus* (SCahmA103), *Trichoderma* (SCahmT105) and Basidiomycete(*P. chysosporium*-9)

2.5 pH values of treated sludge

The values of pH of treated sludge by fungal treatment are illustrated in Fig.4. The pH value varied from 1.8 to 7.5 for 3—9 d of treatment. The pH of fermentation broth was decreased by *Penicillium*, *Aspergillus* and *P. chrysosporium* up to 6 d from its initial value and it was slightly increased after 9 d of incubation except for *Trichoderma* which was followed by a decreasing trend up to 9 d. Most of the strains were growing leading to the excretion of acidic metabolites. The pH recorded in higher solids content of sludge was a higher value than the lower solids content of treated sludge for the case of all strains. The highest pH was recorded in *Trichoderma* (SCahmT105, 7.2) and lowest was in acid producing fungus *Aspergillus* (SCahmA103, pH 1.8) at 6 d of cultivation (Fig.4).

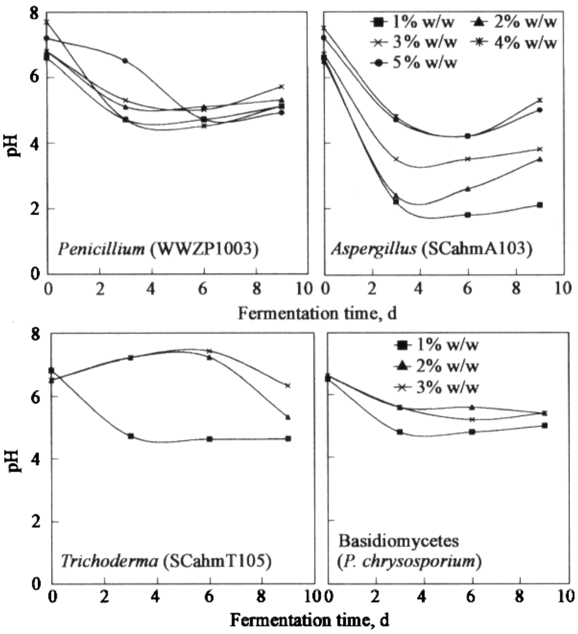

Fig.4 pH values in treated sludge during the fungal treatment of sludge

2.6 Filterability of treated sludge

The filterability test was conducted among the best eight strains of *Penicillium* and *Aspergillus* to evaluate their potentiality by decreasing filtration time of treated sludge as compared to uninnoculated sludge (control). Each four of *Penicillium* and *Aspergillus* were selected for filterability test in second phase experiment. Fig.5 illustrated the results that the filtration time was reduced by the fungal strains for both treatments (4% and 5% w/w) and it was around 12 times shorter in treated sludge than the control (uninnoculated). The growth of fungi entrapped the waste particles and might have accelerated the filterability (Fakhru'l-Razi, 2002b; Hamdi, 1992). The reduction rate of filtration was highly accelerated by fungal treatments after 3 d of fermentation than those of 6 and 9 d. The maximum reduction of filtration time was recorded in strains WWZP1003 (*Penicillium*) and SCahmA103 (*Aspergillus*) while minimum reduction was observed in strains WWZP1008 (*Penicillium*) and SSZA017 (*Aspergillus*) respectively (Fig. 5). The filtration time was 5 times shorter than at the beginning in treated waste of apple distillery waste using *Aspergillus niger* (Friedrich, 1983).

3 Conclusions

It was observed that all of the tested strains were able to grow in wastewater sludge. But the potential strains were screened on the basis of showing their best results in terms of the growth formation (pellets, flocs and filaments), production of biomass, increased TSS%, removal of COD, pH, and filterability test. Observed results showed that the strains WWZP1003, SCahmA103, SCahmT105 and PC-9 (*Phanerochaete chrysosporium*) among the respective groups of *Penicillium*, *Aspergillus*, *Trichoderma*, and Basidiomycete were finally selected for the bioconversion of domestic wastewater sludge. This study indicates that

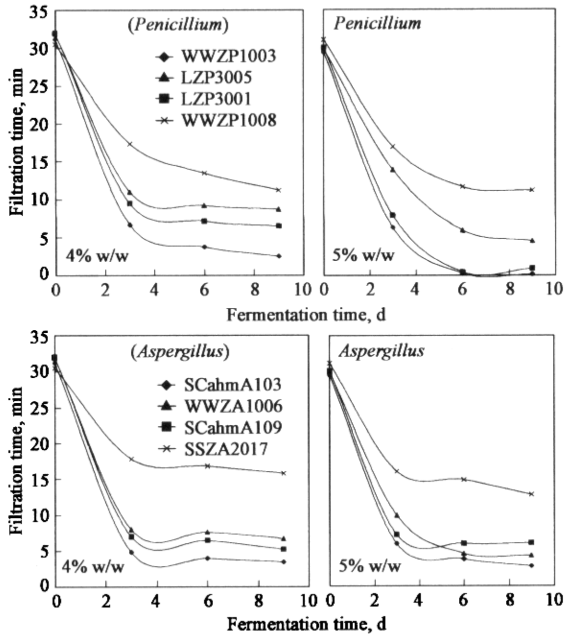

Fig.5 The effect of microbial treatment (*Penicillium* and *Aspergillus*) on filtration in second phase experiment

these potential microbes would accelerate the biodegradation, separation, and filtration process of domestic wastewater sludge by liquid state bioconversion.

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