

EVALUATION OF THE BIODEGRADABILITY OF TAPIOCA STARCH WASTEWATER BY FUNGAL TREATMENT

Watcharapol Wonglertarak¹, Boonchai Wichitsathian^{1,*}, Somchai Dararat², and Jareeya Yimratanabovorn¹

Received: October 07, 2019; Revised: June 02, 2020; Accepted: September 23, 2020

Abstract

In this paper, the performance of the fungal treatment system with various hydraulic retention time (HRT) conditions for improving the biodegradability of tapioca starch wastewater was objectively evaluated. Wastewater used in the study was synthesized using tapioca starch. The experimental process was carried out in Sequencing Batch Reactors (SBR) operated with HRT of 6-48 h, mixed by a diffused aeration system, and adjusted to the optimum pH of 3.0 ± 0.2 . The fungal SBR system produced the effluent with COD, SCOD, and BOD in the ranges of 164-265 mg/L, 36-55 mg/l, and 21-45 mg/L, respectively. This corresponded to the removal efficiency of 83.79-90.31% for COD. The optimal condition for removing organic matter was found to be with the 24hrs HRT. After the fungal treatment, the readily biodegradable (S_s) increased to about 13.10% of TCOD for HRT of 24 h. Furthermore, the BOD/COD ratio in the effluent was 0.275, which is about 1.53 times higher than that in the influent, indicating that the fungi utilization can improve the biodegradable fraction in the wastewater. Applicably, the effluent can be used for agricultural irrigation or discharged into a fishpond.

Keywords: Cassava, starch wastewater, fungal treatment, COD fractionation, sequencing batch reactor (SBR)

Introduction

Industry processes generally produce a large amount of wastewater. Wastewater from industrial agricultural sectors such as the processing tapioca starch or sugar industries mostly consists of organic substances in the forms of proteins (about 40-60%), carbohydrates (25-50%), and fats (about 10%), respectively (Thammaporn, 2012). Cassava starch wastewater normally has a high organic pollutant concentration, high suspended solid concentration,

low pH value, and large load change. The suspended solids mainly in the forms of residual starch and fiber materials are considered to an exorbitant in the cassava starch wastewater. These substances are organic compounds with poor biodegradability (Bo *et al.*, 2011). Conventional biological wastewater treatment usually generates a large amount of low-value bacterial biomass. The treatment and disposal of this excessive bacterial biomass account

¹ School of Environmental Engineering, Institute of Engineering, Suranaree University of Technology, 111 University Avenue, Muang District, Nakhon Ratchasima, Thailand 30000. E-mail: boonchai@sut.ac.th

² Thailand Institute of Scientific and Technological Research, Bangkok, Thailand 10900.

* Corresponding author

for about 40-60% of the operational cost of the treatment plant (Rani *et al.*, 2012). A different form of biomass with a higher value could significantly change the economic aspect of wastewater treatment. Fungi could offer this benefit over bacteria in some selected wastewater treatment processes. Potentially, the biomass from a fungal wastewater treatment has a much higher value than that from the bacterial activated sludge process. Different fungal strains have shown their ability to degrade a wide range of environmental pollutants, i.e., dyes, pharmaceutical compounds, heavy metals, and trace organic contaminants, as well as the ability to degrade lipids, proteins, complex carbohydrates, heteropolymers, aromatic hydrocarbons, and other recalcitrant anthropogenic chemicals by using as sole carbon sources or in co-metabolism, and employing a wide array of extra- and intracellular oxidative, hydrolytic and conjugative enzymes (Blanquez *et al.*, 2008; Cortés-Lorenzo *et al.*, 2016; Maza-Marquez *et al.*, 2016). This paper aims to investigate the performance of a fungal treatment system for the improvement of biodegradability of high carbohydrate wastewater.

Materials and Methods

Synthesis of High Carbohydrate Wastewater

Synthetic high carbohydrate wastewater was prepared using tapioca starch. The synthetic tapioca starch wastewater was heated at $55 \pm 3^\circ\text{C}$ to dissolve particulate matter before feeding it to the system. This was done to simulate high strength particulate wastewater (tapioca starch wastewater) discharge at a high temperature. The components of the synthetic wastewater included 2,000 mg/L of tapioca starch as a sole carbon source, 560 mg/L of NH_4HCO_3 , and 90 mg/L of KH_2PO_4 as a nutrient. Synthetic wastewater constituents are shown in Table 1.

Experimental and Operating Conditions

Enrichment of seed fungi sludge was done by following Racho (2009). The initial seed sludge was isolated from the bottom sediment of an equalization tank from a tapioca starch factory. The enrichment process was carried out by adjusting the naturally mixed fungi and bacteria in the seed sludge

to pH of 3.0 ± 0.2 , which is the optimum value for the mixed fungi to grow and possibly prevent bacterial contamination (Racho, 2009). As a result, three dominant fungal geniuses in the system were included, namely *Trichoderma*, *Aspergillus*, and *Candida*. Presented by several researchers, these fungi have a wide range of enzymes and are capable of metabolizing complex mixtures of organic compounds such as particulate matters and dead cells. The starting experiment was conducted with an initial sludge loading of 1 L. The 7L Sequencing Batch Reactors (SBR) with a working volume of 5 L were set up for the experimental process, and operated by varying HRT from 6 to 48 h (Lei *et al.*, 2014), and by adjusting the value of pH to 3.0 ± 0.2 (Takahiro *et al.*, 2001). Each cycle consisted of four main steps: filling, reacting (aeration), settling, and drawing. The reactors were aerated at different periods to achieve various HRTs. The reactors were mixed and aerated by the compressed air through stone air diffusers to maintain a suitable mixing condition. Each reactor was supplied with sufficient dissolved oxygen ($\text{DO} = 2\text{-}4 \text{ mg/L}$) for microorganism growth, and the room temperature was applied.

Kinetic Evaluation

The oxygen uptake rate (OUR) experiments were conducted following the procedure conducted by Ekama *et al.* (1986) to determine the biodegradability kinetic coefficients of the aerobic heterotrophs. The batch tests were maintained with the temperature $30 \pm 0.5^\circ\text{C}$, pH 3 ± 0.2 , and an addition of the suppressing nitrification of 10 mg N-ammonia/L. The initial ratio of substrate to microorganism (S_0/X_0) was about 1.0-2.0. Suspended solids (MLVSS) concentration (X_0) in the sludge samples was about 300 mg/L for the batch tests. The substrate concentrations (S_0) were varied to govern the quality of the batch respirometric tests. The OUR results were used for calculating the maximum specific growth rates (μ_{max}), substrate utilization rate (r_x), half-velocity constant (K_s), and sludge yield coefficient (Y) based on the Monod kinetics with a regression analysis.

Sampling and Analytical Methods

The influent and effluent samples of the SBR were analyzed following the standard methods for the examination of water and wastewater (APHA *et al.*, 2017). Biochemical oxygen demand (BOD) was determined with an OxiTop®-C measuring pressure head instrument (Expotech USA Inc., Houston, TX, USA). The carbonaceous materials were measured in terms of the COD parameter subdivided into several fractions following Wentzel *et al.* (1999).

Table 1. Characteristics of the synthetic wastewater

Parameter	Unit	Concentration
COD	mg/l	1,693.57
SCOD	mg/l	81.78
BOD ₅	mg/l	304
TKN	mg/l	10.31
NH ₃	mg/l	9.83
BOD/COD	-	0.18
SBOD/SCOD	-	0.15

Results and Discussion

Performance of Organic and Nitrogen Removal

The prepared high carbohydrate wastewater consisted of the quantified solid, organic, and nitrogen contents. The results are presented in Table 1. With a relatively low COD value (1,693 mg/L), the soluble COD to the COD ratio was approximately 4%. It shows that the part is a large organic substance and highly slowly degradation. This wastewater contained a relatively low nitrogen content based on the COD:N ratio, which is likely too low for a conventional biological treatment process. The fungal SBR system produced the effluent with COD, SCOD, and BOD in the ranges of 164-265 mg/L, 36-55 mg/L, and 21-45 mg/L, respectively, implying the removal efficiency of 83.79-90.31% for COD. The HRT of 24 h resulted in the optimal condition for removing organic matters, corresponding to 90.31%, 38.01%, and 85.16% for COD, SCOD, and BOD, respectively, compared to other conditions shown in Table 2 and Figure 1.

This is comparable to the results found by several researchers. Takahiro *et al.* (2001) investigated the treatability of wastewater containing either soluble starch or cornstarch using an internal-loop airlift reactor with *Aspergillus niger* in an open system. For wastewater containing 25g/l cornstarch, the removal efficiency of TOC and the degradation efficiency of starch reached 76% and 99%, respectively. Truong *et al.* (2004) used *Aspergillus oryzae* for the treatment of the cassava starch processing wastewater in the forms of both pellets and freely dispersed hyphal elements at pH 3.0. The treatment efficiencies obtained for TOC, COD, and starch after 96 h were 72%, 75%, and 77%, respectively.

The nitrogen removal efficiency in terms of TKN for all conditions of HRT was low. This is probably due to the presence of a toxic environment for nitrifying bacteria when the pH value was adjusted to about 3.0 ± 0.2 . The pH for the optimal growth of nitrosomonas and nitrobacter bacteria was in the ranges of 6.0 to 9.0 and 6.3 to 9.4, respectively. However, after treating by fungi, high organic contents were hydrolyzed to low organic contents. Compared to other conditions in Table 2 and Figure 2, the optimal condition for nitrogen

removal was 24 h HRT, and the removal efficiencies were found to be 11.4% and 24.16% for TKN and NH_3 , respectively.

The effluent from the treatment with fungus then can be used for agricultural irrigation or discharged into the fishpond. However, the effluent pH was approximate 3.4-4.2, and it should be increased the value by applying NaOH or CaCO_3 for further uses. Following the requirements of local environmental administration, the effluent of wastewater treatment station should reach the GB5084-92 "irrigation water quality standards", which are $\text{COD} < 200 \text{ mg/L}$, $\text{BOD}_5 < 80 \text{ mg/L}$, $\text{SS} < 250 \text{ mg/L}$ and the pH value is 5.5-8.5 (Bo *et al.*, 2011).

Biodegradability and COD Fractionations

The biodegradability and COD fractionations were evaluated, and the results are illustrated in Figure 3. The ratio of BOD/COD is often used as an index to evaluate the biodegradability of wastewater. The $\text{BOD}/\text{COD} > 0.45$ indicates that the biodegradability is very good; for $\text{BOD}/\text{COD} = 0.45$, the biodegradability is good; for $\text{BOD}/\text{COD} = 0.2-0.3$, the biodegradability is poor; for

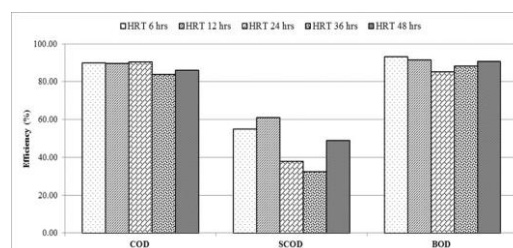


Figure 1. Efficiency organic removal of fungal treatment at various HRT

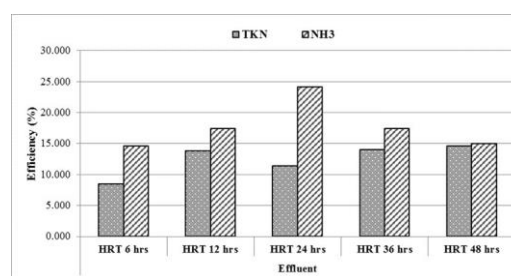


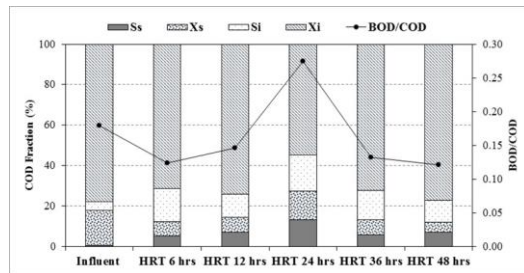
Figure 2. Efficiency nitrogen removal of fungal treatment at various HRT

Table 2. Water quality of high carbohydrate wastewater treated by fungal

Condition	COD (mg/l)	SCOD (mg/l)	BOD (mg/l)	TKN (mg/l)	NH_3 (mg/l)
Influent	1693.56	81.77	304.00	10.30	9.83
Effluent	HRT 6 h	170.48	36.87	21.10	9.43
	HRT 12 h	173.91	31.929	25.40	8.87
	HRT 24 h	164.10	50.69	45.10	9.10
	HRT 36 h	274.49	55.16	36.20	8.85
	HRT 48 h	235.31	41.81	28.50	8.77

Table 3. Comparison of kinetic coefficients in wastewater treatment systems

System	Culture	$\mu_{\max}$ (d ⁻¹)	K_s (mg/L)	Y (mg MLVSS/mg COD)	Ref.
Batch	<i>Aspergillus niger</i>	2.33	3,450	-	Poulsen (2005)
Batch	<i>M. alpina</i>	1.13	-	-	Xue <i>et al.</i> (2018)
DHS	Mix culture	1.6-3.4	52-100	0.63	Racho (2009)
SBR	Mix culture	2.66	53.69	0.31	This study

**Figure 3. The COD fractionation and biodegradability of fungal treatment at various HRT**

BOD/COD < 0.2, the biological treatment is unsuitable. The BOD/COD ratio of high carbohydrate wastewater was found to be about 0.18 that is very low. The COD fractionations were evaluated for the potential biodegradability. The slowly biodegradable organic matter (X_s) was hydrolyzed to the readily biodegradable organic matter (S_s) and inert soluble organic matter (S_i). The influent contained a particular inert (X_i) fraction and readily biodegradable (S_s) of about 77.93% and 0.71% of TCOD, respectively. The high carbohydrate wastewater has a high molecular weight that is hardly biodegradable by heterotroph bacteria. After the treatment, the readily biodegradable organic substrate (S_s) increased at different HRT conditions and showed the maximum value for HRT of 24 h, which was about 13.10% of TCOD. This showed that the hydrolysis from X_s into S_s and S_i occurred more at the optimal condition, implying more efficient for the removal of organic matter. The X_i fraction (inert particulates) exits the plant and binds up in sludge flocs (Henze, 2002). Although the X_i fraction is inert, it can be removed by biological treatment (Fall *et al.*, 2012). Furthermore, the effluent provided the BOD/COD ratio of 0.275, which is 1.53 times higher than that of the influent, indicating that the biodegradable fraction can be increased through the fungus utilization. The biodegradability kinetics of tapioca starch wastewater with a fungal treatment is shown in Table 3. The highest specific growth rate ($\mu_{\max}$) of microorganisms was 2.66 per day. Moreover, it can also be observed that the yield (Y) of the fungal culture was 0.31 mg VSS/mg COD removed. This is probably due to an increase of the microorganism

growth to uptake substrates in a more easily biodegradable form. This is consistent with the removal efficiency of organic matter in terms of COD, TS, and VS.

Conclusions

The application of fungi for treating tapioca starch wastewater possibly provides the effective removal of COD in the range of 83-90%. For the SBR system with HRT of 24 h, the effluent had the BOD/COD ratio of 0.275 higher than that of the influent. This suggests that the biodegradable fraction was improved in the presence of fungi. The readily biodegradable (S_s) increased to about 13.10% of TCOD. An increase of slowly biodegradable hydrolysis into readily biodegradable was also observed. The single-stage process developed in this study can reduce the operation costs because the initial cassava starch wastewater properties provide the selected fungus favorable growth conditions with the initial pH of 3.0-5.0. Contamination may be eliminated at a low pH. Plus, the fungal biomass contains a high protein content and is safe for human and animal consumption, so it is marketable and can offset the operating costs. Therefore, this process is technically feasible for the treatment of any starch processing effluent with by-product recovery, contributing to sustaining the global management of environmental resources.

Acknowledgments

This work was supported by Thailand Institute of Scientific and Technological Research and Suranaree University of Technology and Thailand Institute of Scientific. We would like to thank the staff for their continual assistance in preparing and carrying out this research in the Institute of Environmental Engineering, Suranaree University of Technology.

References

- APHA, AWWA, and WEF. (2017). Standard methods for the examination of the water and wastewater. Washington D.C. : American Public Health Association.

- Poulsen, B.R. (2005). Characterization of pathway engineered strains of filamentous fungi in submerged cultures. Ph.D. thesis Wageningen University, Wageningen, Netherlands.
- Blanquez, P., Sarra, M., and Vicent, T. (2008). Development of a continuous process to adapt the textile wastewater treatment by fungi to industrial conditions. *Process Biochem.*, 43:1-7.
- Bo, Y., Kanghuai, L., Cheng, S., Wenyu, Z., Jiagang, S., Fei, L., and Yinjie, W. (2011). Compared selection of pretreatment technology for cassava starch wastewater treated by anaerobic process. *International Symposium on Water Resource and Environmental Protection.*, 1:1,533-1,536.
- Cortés-Lorenzo, C., González-Martínez, A., Smidt, H., González-López, J., Rodelas, B. (2016). Influence of salinity on fungal communities in a submerged fixed bed bioreactor for wastewater treatment. *Chem. Eng. J.*, 285:562-572.
- Ekama, G.A., Dold, P.L., and Marais, G.V.R. (1986). Procedures for determining influent cod fractions and the maximum specific growth rate of heterotrophs in activated sludge systems. *Water Sci. Technol.*, 18:91-114.
- Fall, C., Millán-Lagunas, E., Bâ, K.M., Gallego-Alarcón, I., García-Pulido, D., Díaz-Delgado, C., and Solís-Morelos, C. (2012). COD fractionation and biological treatability of mixed industrial wastewaters. *J. Environ. Manage.*, 113:71-77.
- Lei, Z., Guang-Li, C., Ai-Jie, W., Hong-Yu, R., and Nan-Qi, R. (2014). An anaerobic sequential batch reactor for enhanced continuous hydrogen production from fungal pretreated cornstalk hydrolysate. *Int. J. Hydro. Energy.*, 39:19,311-19,316.
- Henze, M., Harremoes, P., Jansen, J.L.C., and Arvin, E. (2002). *Wastewater Treatment: Biological and Chemical Processes*. 3rd ed. Springer, N.Y., 422p.
- Maza-Marquez, P., Vilchez-Vargas, R., Kerckhof, F.M., Aranda, E., Gonzalez-Lopez, J., and Rodelas, B. (2016). Community structure, population dynamics and diversity of fungi in a full-scale membrane bioreactor (MBR) for urban wastewater treatment. *Water Res.*, 105:507-519.
- Racho, P. (2009). Investigation of downflow hanging sponge (DHS) system using bacterial and fungal cultures as a post treatment for the UASB effluent of a tapioca starch wastewater, [Ph.D. thesis]. Suranaree University of Technology. Nakhonratchasima, Thailand.
- Rani, R.U., Kumar, S.A., Kaliappan, S., Yeom, I.T., and Banu, J.R. (2012). Low temperature thermo-chemical pretreatment of dairy waste activated sludge for anaerobic digestion process. *Biores. Technol.*, 103:415-424.
- Thammaporn, S. (2012). Application of fungal downflow hanging sponge in combination with bacterial downflow hanging sponge systems treating high carbohydrate wastewater, [Master thesis]. Suranaree University of Technology. Nakhonratchasima, Thailand.
- Takahiro, S., Naoyuki, M., and Keisuke I. (2001). Treatment of high-strength wastewater containing cornstarch by internal-loop airlift reactor with *aspergillus niger* pell. *Japanese J. Water Treat. Bio.*, 37 (3):111-116.
- Truong, Q.T., Naoyuki, M., and Keisuke, I. (2004). Growth of *aspergillus oryzae* during treatment of cassava starch processing wastewater with high content of suspended solids. *J. Biosci. Bioeng.*, 97(5):329-335.
- Wentzel, M.C., Mbewe, A., Lakay, M.T., and Ekama, G.A. (1999). Batch test for characterization of the carbonaceous materials in municipal wastewaters. *Water SA*, 25(3):327-336.
- Xue, F., Genhai, Z., Jun, D., Hui, L., Peng, W., Li, W., Junying, S., and Zhiming, Z. (2018). Macro-morphological characterization and kinetics of *Mortierella alpina* colonies during batch cultivation. *PLoS ONE*, 13(8):1-11.