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Pilot regulation of MnP-SA for treating PTA wastewater

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Abstract: In the pilot study of treating the purified terephthalic acid(PTA) wastewater with the functional Strain Fhhh in the carrier activated sludge process(CASP), the ratio of COD : TN : TP and the concentrations of Cu, Mn, Se and Zn were controlled to improve the manganese peroxidase(MnP) levels for increasing the treatment efficiency. When the ratio of COD:TN:TP was 100:0.36:0.15 and the concentrations of Cu, Mn, Se and Zn were 0.54, 5.07, 0.00 and 0.08 mg/L, the MnP specific activity(MnP-SA) reached 689 U/L, and the sludge loading rate to COD(SLRC) was 1.09 d^{-1}, which was 4—7 fold of that in other processes reported. The data indicated that improving MnP level could enhance the degradability of Fhhh. And the potentials of Fhhh and CASP will be also discussed in this paper.

Keywords: functional strain; PTA wastewater; pilot engineering; MnP-SA; regulation; SLRC

Introduction

Purified terephthalic acid(PTA) is a common saying of terephthatic acid(TA), it is widely used as a material in chemical synthesis. Over 70% of COD in PTA wastewater comes from aromatic pollutants including dimethylterephthalate, benzoic acid, p-toluic acid, phthalic acid, terephthalic acid and 4-carboxybenzaldehyde (Fajardo, 1997). Most of the aromatic pollutants existing in PTA wastewater are persistent organic pollutants (POPs) and recalcitrant to degradation by native microorganisms. The POPs and their derivatives in environment can produce the toxicities of carcinogenesis, mutagenesis and teratogenesis (Lemini, 1995; Pavan, 2001; Jha, 1998; Ema, 1997). The currently used biological processes for PTA wastewater treatment still meet the problems, such as long treatment time needed, highly energy consumed and low efficiency appeared.

The functional strain and the optimized process are the two key problems required to be solved at present for improving the efficiency in treatment of PTA wastewater. They are particularly in urgent need now(Guan, 2002).

The functional strain Fhhh, the start strain of the pilot study, was constructed through the protoplast fusion of three parental strains and patented in 2002(Cheng, 2002). And the PTA wastewater treatment process, carrier activated sludge process (CASP), was used in this research. The CASP was regulated in the ratio of COD : TN : TP and the concentrations of Cu, Mn, Se and Zn to optimize the expression of manganese peroxidase (MnP) for enhancing treatment efficiency. The three parental strains of Fhhh were *Phanerochaete chrysosporium* (PC), *Saccharomyces cerevisiae* (SC) and the native bacterium YZ1. Fhhh could integrate the useful characters, high degradability from PC, high flocculation from SC and high adaptability from YZ1, demonstrated by Zhong(Zhong, 2000).

POPs degradation by microorganisms mainly depends on the conditions under which enzyme secrets and enzyme reacts. If the conditions could not meet the requirements of enzymatic gene expression and enzymatic degradation reaction, functional strain may lose the function for degradation of POPs in wastewater. The *mnp* gene existing in Fhhh cell came from its parental strain PC. The expression production of *mnp* gene is MnP. MnP is a series of extracellular enzymes and is very important for the degradation of POPs. The expression of MnP at a high level can be achieved under some conditions (Kuwahara, 1984; Yan, 2004).

This pilot study was conducted from Nov. 2003 to Jun. 2004 for 180 d. Its results involved the ratio of COD:TN:TP and the concentrations of Cu, Mn, Se and Zn regulated. The data showed that the manganese peroxidase specific activity (MnP-SA) of Fhhh was 689 U/L while its sludge loading rate to COD(SLRC) was 1.09 d^{-1} and it was 4—7 fold of that reported in other processes. It is possible to increase the PTA wastewater treatment efficiency through the regulation of the conditions for the expression of MnP and optimization of CASP with Fhhh.

1 Materials and methods

1.1 Start strain and medium

Fhhh patented was the start strain in this pilot study (Cheng, 2002). The parental strains, *P. chrysosporium* was supplied by Prof. Wang, *S. cerevisiae* was obtained from Dongguan Sugar Mill, and the native bacterium YZ1 was screened from the activated sludge of PTA wastewater treatment plant of NJYZ. The medium for fungi cultivation was used(Zhu, 1993).

1.2 Enzymatic assay

Ammonium tartrate was the TN source, NaH_2PO_4 was the TP source and the metal elements of Cu, Mn, Se and Zn were the chlorides(AR). They were used for regulating the ratio of COD:TN:TP and the concentrations of Cu, Mn, Se and Zn.

PTA wastewater sample was the mixed liquor taken from the aeration tank of CASP of the pilot engineering at NJYZ. It was centrifuged at 3000 r/m in for 20 min to remove cells and other suspended solid. The supernatant of the wastewater sample was utilized as the MnP resource in the following enzymatic assay.

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MnP-SA levels were measured with spectrophotometer at λ 469 nm according to Gold (Gold, 1988) and 2, 6-dimethoxyphenol (DMP, USA-Aldrich product) was the substrate of MnP reaction. One MnP activity unit was identified 0.001 ΔA_{469} in this research and one MnP-SA unit was equal to 1 U/L.

1.3 Flow scheme of CASP in pilot engineering

The flow scheme of CASP with Fhhh in the pilot study is shown in Fig.1.

1.4 Measurement of the wastewater quality and CASP parameters

The concentrations of COD_{Cr}, BOD_5, MLSS, SV, SVI and DO were measured according to APHA (APHA, 1985),

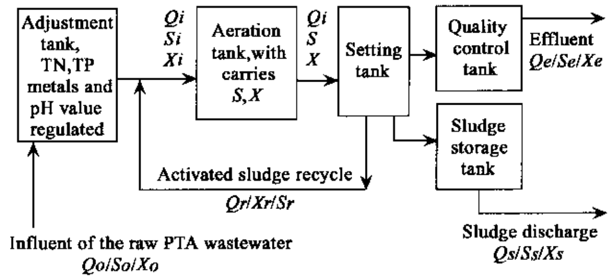

Fig.1 The flow scheme of CASP with Fhhh in PTA wastewater treatment

metal elements detected with ICP-J-A-1100 USA and TOC examined by TOC-5000, Shimadzu Corporation (Table 1).

Table 1 Results of CASP and PTA wastewater quality analyzed

Parameter	Value	Parameter	Value	Parameter	Value
Qo, m^3/d	4.8—7.2	*Sr*, g/L	0.122—0.727	*SA* = *θc*, d	4.3—38.0
So, g/L	1.819—4.717	*Xr*, g/L	2.180—4.520	*HRT* = *θ*, d	1.37—2.05
Xo, g/L	0.00—0.00	*Qe*, m^3/d	0.8—6.2	BOD_5, g/L	1.219—3.164
Qi, m^3/d	9.6—14.4	*Se*, g/L	0.122—0.727	TOC, g/L	0.565—1.465
Xi, g/L	1.040—2.260	*Xe*, g/L	0.085—0.372	TSS, g/L	0.092—0.320
Si, g/L	0.971—2.722	*Qs*, m^3/d	1—4	DO, mg/L	0.16—5.64
X, g/L	1.090—2.260	*Ss*, g/L	0.122—0.727	*T*, ℃	25 ± 5
S, g/L	0.122—0.727	*Xs*, g/L	2180—4520	*SV*, %	82.78
Qr, m^3/d	4.8—7.2	*V*, m^3	9.84	*SVI*, ml/g	456

2 Results and discussion

2.1 Correlation between MnP-SA levels and the three loading values

In Table 2, the data obtained from the pilot engineering from May 10 to June 10 of 2004 at NJYZ. The levels of MnP-SA of Fhhh in the mixed liquor of the aeration tank of CASP were tested for 9 times. The values of MnP-SA are listed in the first column on the left of Table 2.

Table 2 Correlation between MnP-SA levels and the three loading rates

MnP-SA, U/L	kgCOD/(kg·d)	kgTOC/(kg·d)	kgCOD/(m^3·d)
139 ± 3.6	0.72	0.21	1.14
146 ± 3.6	0.95	0.29	1.74
300 ± 9.5	0.52	0.15	0.96
433 ± 4.8	0.77	0.22	1.39
562 ± 6.3	1.23	0.37	1.34
576 ± 4.8	0.85	0.26	1.84
867 ± 6.3	1.46	0.45	3.26
851 ± 6.5	1.16	0.36	1.86
836 ± 11.5	1.09	0.34	1.74
r value	$n = 9$, $r = 0.730$	$n = 9$, $r = 0.746$	$n = 9$, $r = 0.636$

Three loading parameter of Fhhh are shown in Table 2, the sludge loading rate to COD removed (SLRC), the sludge loading rate to TOC removed (SLRT), and the volumetric loading rate to COD removed (VLRC).

Table 2 shows that positive correlations existed between the levels of MnP-SA are of Fhhh and the values of SLRC, SLRT and VLRC ($r = 0.730$, 0.746 and 0.636) which were demonstrated by the statistics examinations ($r_{(0.05)9} = 0.5822$, $r > r_{0.05(9)}$, $P < 0.05$). This means that the higher the levels of MnP-SA are, the higher the three loading rates will be. Improving the level of MnP-SA of Fhhh could increase the sludge loading rate to COD and TOC, and could also enhance the volumetric loading rate to COD in aeration tank.

The three models might indicate the positive correlations quantitatively between the levels of MnP-SA of Fhhh and the values of SLRC, SLRT and VLRC respectively. The three models are $Y = 0.000731X + 0.589$ for the calculation of SLRC value, $Y = 0.000242X + 0.168$ for SLRT and $Y = 0.00146X + 0.931$ for VLRC on the basis of MnP-SA value obtained. In the three models, X is the level of MnP-SA of Fhhh and Y is the value for one of the three loading rates, SLRC, SLRT and VLRC.

2.2 Effects of COD : TN : TP and metal levels on the expression of MnP

In Table 3, the values of MnP-SA of Fhhh were obtained from the four reaction systems during 180 d of the pilot engineering. The four PTA wastewater degradation systems with Fhhh varied in the ratios of COD : TN : TP and the concentrations of the four metals after regulation. The purposes of the control of the ratios of COD : TN : TP and the concentrations of the four metals, Cu, Mn, Se and Zn, were to optimize CASP and to improve the MnP expression levels so as to increase the PTA wastewater treatment efficiency.

In Table 3, Type Ⅰ was the raw PTA wastewater reaction system. The mean value of MnP-SA of Fhhh was 75 U/L tested in Nov. 2003 in the pilot engineering.

Type Ⅱ was the raw PTA wastewater regulated, in which the metal levels were controlled only during the May 2004 in the pilot engineering. The mean value of MnP-SA was 195 U/L.

Type Ⅲ was the raw PTA wastewater regulated for both the ratios of COD : TN : TP and the concentrations of the metals during the May to Jun. 2004 in the pilot engineering. The mean value of MnP-SA was 689 U/L.

Type Ⅳ was the raw PTA wastewater regulated optimally for the expression of MnP at the highest level, and the values of COD:TN:TP and the metals were both controlled in Jun. 2003 in the laboratory. The mean value of MnP-SA was 1200 U/L.

Table 3 Effects of the values of COD:TN:TP and the metals on MnP-SA levels

Systems	Type Ⅰ	Type Ⅱ	Type Ⅲ	Type Ⅳ
MnP-SA, U/L	75	195	689	1200
COD, mg/L	2648	2243	3855	2648
TN, mg/L	1.20	1.20	14.07	11.78
TP, mg/L	2.48	2.48	5.64	2.25
COD:TN:TP	100:0.05:0.09	100:0.05:11	100:0.36:0.15	100:0.44:0.09
Cu, mg/L	< 0.006	0.75	0.75	5
Mn, mg/L	3.200	4.94	4.94	10
Se, mg/L	0.00	0.00	0.00	0.05
Zn, mg/L	< 0.010	0.14	0.14	0.26

Table 3 reveals a gradient of the MnP-SA levels for the four reaction systems, that is Type Ⅳ > Type Ⅲ > Type Ⅱ > Type Ⅰ. This gradient means that the levels of MnP-SA of Fhhh in PTA wastewater could be improved and the treatment efficiency should be enhanced.

There was a ratio among the MnP-SA values of the 4 reaction systems that was Type Ⅳ : Tpye Ⅲ : Type Ⅱ : Type Ⅰ = 16 : 9 : 3 : 1. It could be concluded that the level of MnP-SA in the optimal reaction system (Type Ⅳ) was 16 fold of that of the unregulated reaction system (Type Ⅰ).

2.3 Judgment of the feasible concentrations of metals

Table 4 shows the Type Ⅳ was the optimal system for the expression of MnP, it could not be used in engineering actually, because 5 mg/L of Cu and 10 mg/L of Mn needed in Type Ⅳ are higher than the threshold values listed in the wastewater discharge standard (GB8978-1996) which are 2 mg/L of Cu and 5 mg/L of Mn.

Table 4 The feasible concentrations of metals in treatment engineering

Metals	Cu, mg/L	Mn, mg/L	Se, mg/L	Zn, mg/L
In (GB8978-1996)	2.0	5.0	0.50	5.0
In Type Ⅲ	0.75	4.94	0.00	0.14
In Type Ⅳ	5.0	10	0.05	0.26
Feasible concentration	2.0	5.0	0.05	0.26

Comparison of the four metal concentrations in (GB8978-1996) with those in Type Ⅳ system, the feasible concentrations of the four metals in the treatment engineering actually should be 2.0 mg/L of Cu, 5.0 mg/L of Mn, 0.05 mg/L of Se and 0.26 mg/L of Zn shown in the last line of Table 4.

The feasible concentrations of the four metals stay within the range of the wastewater discharge standard (GB8978-1996) and will be closer to those in Type Ⅳ than in the other three reaction systems. And the MnP expression level in the feasible system will be also higher than in Type Ⅰ, Type Ⅱ and Type Ⅲ.

2.4 The potentials of Fhhh and CASP

In Table 5, the value of the Fhhh SLRC was 1.09 d^{-1}, it was measured in the Type Ⅲ reaction system in the pilot PTA wastewater treatment. The value of 1.09 d^{-1} is 7 fold of that in the upflow anaerobic sludge blanket (UASB) reported by Kleerebenem (Kleerbenem, 1997), 4 fold of that in UASB reported by Cheng (Cheng, 1997) and 5 fold of that in the aeration/oxidation (A/O) system reported by Zhao (Zhao, 1994). The data might illustrate the functional strain Fhhh and the pilot engineering CASP have the potentials obviously useful in PTA wastewater treatment.

Table 5 shows that the VLRC value of Fhhh is lower than that reported in two processes (Kleerebenem, 1997; Cheng, 1997), because the biomass of Fhhh was 1.68 g/L only, lower than that of other processes reported. In fact, when the chemical fiber carrier was fixed in the aeration tank of CASP, the Fhhh biomass could reach 6 g/L while the value of VLRC for Fhhh could reach 6.82 gCOD/(L·d), higher than the other three processes reported as well as the value of SLRC.

Table 5 Comparison of characters between the pilot study and other process

Reference	Kleerebezem, 1997	Cheng, 1997	Zhao, 1994	This paper
Treatment process	UASB	UASB	A/O	CASP
Reactor, L	0.2	19	200	9840
COD, g/L	3.5	4.0	1.1	3.3
Running time, d	120	60	120	180
VSS/MLSS, g/L	2.6 (VSS)	10.80 (VSS)	3.30 (MLSS)	1.68 (MLSS)
HRT, d	1	1.8	1.67	1.71
VLRC, g/(L·d)	3.9	2.74	0.66	1.91
SLRC, g/(g·d)	0.15	0.26	0.20	1.09

3 Conclusions

MnP specific activity level of Fhhh was correlated with levels of SLRC, SLRT and VLRC positively.

Type Ⅲ was a better system for the expression of MnP in the pilot engineering, but not the best. Its mean value of MnP-SA of Fhhh was 689 U/L.

The SLRC value of Fhhh in the pilot engineering for PTA wastewater treatment was 4—7 fold of that in other treatment processed reported.

The VLRC value of Fhhh may reach 6.82 gCOD/(L·d) because the biomass can be raised to 6 g/L in the aeration tank of CASP.

Terminology

Symbol	Meaning	Symbol	Meaning
CASP	Carrier-activated sludge process	*Qo*	Flow of the raw PTA wastewater
COD_{Cr}	Chemical oxygen demand, COD_{Cr} = COD	*So*	COD in the raw PTA wastewater
BOD_5	Biochemical oxygen demand	*Xo*	Biomass in the raw PTA wastewater, $Xo = 0$
DO	Dissolved oxygen	*X*	Biomass in the aeration tank
HRT	Hydraulic retention time, $HRT = \theta$	*S*	COD in the aeration tank
MLSS	Mixed liquor suspended solid	*Qr*	Return flow of activated sludge
MnP-SA	Specific activity of MnP	*Sr*	COD in *Qr*
SA	Sludge age, SA = θc	*Xr*	Biomass in *Qr*
SLRC	Sludge loading rate to COD	*Qe*	Flow of wastewater discharged finally
SLRT	Sludge loading rate to TOC	*Se*	COD in *Qe*
SV	Sludge volume	*Xe*	Biomass in *Qe*
SVI	Sludge volume index	*Qs*	Flow of activated sludge discharged
PTA	The purified terephthalic acid, PTA = TA	*Ss*	COD in *Qs*
TOC	Total organic carbon	*Xs*	Biomass in *Qs*
TSS	Total suspended solid	*Qi*	Influent flow of the aeration tank,
V	Effective volume of aeration tank	*Si*	COD in *Qi*
VLRC	Volumetric loading rate to COD	*Xi*	Biomass in *Qi*

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