Application of energy spilling mechanism by *para*-nitrophenol in biological excess sludge reduction in batch-activated sludge reactor

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Abstract

Reduction of biomass production coefficient is an ideal solution for the reduction of excess sludge especially in industrial wastewater treatment plants. Studies were carried out in two sequencing batch reactors, which were controlled online. After presenting a stable situation in reactors, during 24 months of the study, sampling and examining of chemical oxygen demand (COD), biochemical oxygen demand, pH, sludge volume index (SVI), specific oxygen uptake rate (SOUR), remaining *p*NP, and biomass yield (*Y*) were implemented.

Results have shown that among different retention times (5, 10, 15, 25 days), maximum COD removal efficiency (95%) was achieved in 10 days, without bulking and foaming problems. In 10 days of sludge retention time, average *Y* and kinetic coefficient (K_d) were calculated: 0.58 mg biomass/mg COD and 0.058 1/day, respectively, and correlation coefficient (R^2) was 0.98. Different concentration of *p*NP were used due to energy spilling effect in the reactor and the results show that injection of 100 mg/L *p*NP to the reactor can reduce synthetic coefficient *Y* from 0.58 to 0.27 mg biomass/mg COD without *p*NP injection, so that the excess sludge was reduced by 0.56%. Although, an increase of 193 mg/L soluble COD in the effluent was observed. On the other hand, in this concentration of *p*NP, SOUR rate reached 31 mg O_2/h/g volatile suspended solids, and SVI rate reached less than 48 mL/g. In the concentration of 150 mg/L *p*NP, no sludge was produced, but COD rate of the effluent increased to 480 mg/L. Otherwise, *p*NP rate is an environmental limitation in effluent and sludge disposal.

Keywords

Sequencing batch reactor, Biological sludge, Biomass yield, COD, *Para*-nitrophenol

Background

Removal of organic materials by biological oxidation is a core technology in wastewater treatment process. New cells (sludge), carbon dioxide, soluble microbial products, and water are the end products for this process. The activated sludge process is widely used for municipal and industrial wastewater treatment and generates a large quantity of excess sludge daily. So far, the ultimate disposal of excess sludge has been and continues to be one of the most expensive problems faced by wastewater utilities, e.g., the treatment of the excess sludge may account for up to 65% of the total plant operation cost. So in recent years, increased attention has been given to the minimization of waste sludge in wastewater treatment process [1-6].

Reduction of biomass production in wastewater treatment was discussed when costs and difficulties of treatment and disposal of the sludge were considered. On the other hand, new rules and severe standards of reuse and disposal of the sludge about different organic and inorganic pollutants and pathogens forced wastewater treatment experts to invent aerated biological treatment methods which produce less sludge amount. In other words, if the problem of excess sludge production is solved, most of the problems would also be solved considerably in the treatment and disposal of the biomass [7,8]. Due to severe problems and heavy costs of operations of sludge treatment, mechanisms of biomass reduction were considered in the recent years. A collection of effective determinations in this field are:

- Self-destructive process [9-12]
- Uncoupled metabolism using OSA process [5,12-18]
- Increasing soluble oxygen of aeration pond [19]
- Oxidation of a part of sludge by chlorine or ozone [2,5,7,8,18,20-23]
- Increasing temperature in returned sludge to the reactor [6,24-26]
- Energy spilling by compounds resistant to degradation and toxicant [3-5,17,27-30]
- pH changes [11,24]
- Using electrical pulse in returned sludge [31]
- Using ultrasonic waves in returned sludge [32]
- Using bacteriophages such as protozoa and metazoan [33,34]

For most of the aerobic bacteria, adenosine-5′-triphosphate (ATP) is generated by oxidative phosphorylation, in which process electrons are transported through the electron transport system from a source of electrons at elevated energy levels (substrate) to a final electron acceptor (oxygen). The chemiosmotic theory shows that the oxidative phosphorylation is driven by proton gradient built up across cell membrane [11]. However, the tight coupling of respiration and phosphorylation can be disturbed by molecules known as metabolic uncouplers. In the presence of metabolic uncouplers, the energy generated from the oxidation of organic substrate would be lost as heat rather than being captured in ATP. As a result, the growth efficiency is much lowered in uncoupler-containing microbial culture. Metabolic uncouplers include a diverse group of molecule-structures, but they are all lipophilic weak acids [5,29,35,36] many of which have been used to reduce excess sludge production from the activated sludge processes, such as nitrophenol, chlorophenol, 3;3′,4′,5-tetrachlorosalicylanilide (TCS), 2,4,5-trichlorophenol (TCP), carbonyl cyanide-p-trifluoromethoxyphenyl hydrazone, cresol, aminophenol, and so on [3,4,20,27-29,37,38].

In 1998, Mayhew and colleagues found that injecting 35 mg/L of 2,4 dinitrophenol to the activated sludge reactor in 20°C, 0.3 mg mixed-liquor suspended solids (MLSS)/g chemical oxygen demand (COD) in pH = 7, and 2.5 g/L MLSS, sludge retention time (SRT) = 15 days and hydraulic retention time (HRT) = 5.5 h can reduce biological excess sludge, but the only problem is a 3.7% COD increase in the effluent [38]. In 1998, Low et al., found that continuous injection of 100 mg/L *para*-nitrophenol (*p*NP) to a culture media containing pseudomonas at 30°C and pH = 6.2 to 7 can reduce biological excess sludge from 62% to 70% [28]. In 1999, Strand and colleagues found that a 2 to 2.5 mg/L increase of TCP to the activated sludge continuously cultivated at 20°C and pH = 7, MLSS = 2.5 g/L, SRT = 5 days, HRT = 3.5 h can reduce biological excess sludge about 50% [39]. In 2000, Low reported that in a lab-scale-activated sludge system, 49% of the biomass production would be reduced by injecting 100 mg/L *p*NP in the culture media. The COD removal efficiency was reduced about 25%. No sludge production was observed when 120 mg/L *para*-nitrophenol was added [29].

In 2003, Yang et al., found that 20 mg/L m-chlorophenol (mCP) injection to non-continuous activated sludge reactor at 25°C, pH = 7 can reduce biological excess sludge 86.9%, but there is the problem of 13.5% increasing of COD in effluent [40].

In 2003, Chen et al., found that efficiency of cellular growth or sludge production can reduce excess sludge about 78% in a non-continuous medium by injecting 0.8 mg/L of TCS at 20°C and pH = 7 but is ineffective in COD removal. In fact, most of the organic substrates would be oxidized by energy spilling mechanism, and cell synthesis rarely happens in the presence of TCS combined within the medium [15]. In 2007, Chase et al. indicated that at the dosage of 100 mg/L *p*NP in the activated sludge stage, the reduction of excess sludge was 16% and 27% of TSS in the case of wastewater handling units fed with raw and settled wastewater, respectively [41].

Although the reduction of sludge production happens by adding degradation-resistant materials and toxicants, reduction of pollutants to an acceptable value in effluent is necessary due to environmental standards. This method increases the cost of biological treatment system operation but is an acceptable way for a stronger industrial wastewater treatment. Therefore, this study was conducted to investigate and analyze the influence of energy spilling mechanism by *para*-nitrophenol in biological excess sludge reduction on the activated sludge in a batch culture process.

Methods

Collection of samples and wastewater characteristics

Two non-continuous cylinder-shaped reactors made of Plexiglas were used for this study with 25-cm internal diameter, 60-cm height, 20-L working volume, and 10-L treatment capacity in each cycle. Figure 1 shows a pilot plant view of non-continuous sequencing reactors. Planning system operation was done by software. The software was able to control and save all operations of the system.

Figure 1 Sequencing batch reactor (SBR) pilot plant.

Pilot sewage input was made of 40 gr of industrial milk powder and 100 L drinking water. In this study, quality profile of the synthetic wastewater was as below:

COD = 600 mg/L
Biological oxygen demand (BOD_5) = 420 mg/L
Nitrous nitrogen concentration = 4.7 mg/L nitrogen
Ammoniac nitrogen concentration = 0.7 mg/L
Organic nitrogen concentration = 30 mg/L
Kejeldal nitrogen concentration = 30.7 mg/L
Phosphorus concentration= 10.5 mg/L

Pilot start-up

For setting up and starting up the sequencing batch reactor (SBR), reverse activated sludge of Ekbatan wastewater treatment plant was used as seed - which did not have any bulking, foaming, and pinpoint flock - in about 2 L for each SBR pilot reactors with a total volume of 20 L, and synthetic wastewater with COD of 600 ± 20 mg/L was added to the reactor.

According to the type and characteristics of the used wastewater in the present work, time duration was the same in both the reactors. Filling ends in 3 min, aeration in 4 h, sedimentation in 1 h 45 min, and draining in 12 min, and in fact, the required time for filling was shorter and approximately took place in 1 min and 10 s.

Aeration and reaction took place approximately 2 weeks for flock or biomass formation in such a way that only the reaction was done, but nutrients were added to the reactor daily. After 2 weeks, the pilot system of SBR was launched by 5 cycles which are filling, reaction, discharge, sludge discharge, and rest. COD, suspended solids (SS), pH of the effluent in different turns were very close, which showed that reactor start-up came to its end. After having stable conditions in the reactors during 12 months of study, sampling, and testing parameters such as COD, MLSS, MLVSS, dried sludge solid percentage, sludge volume index (SVI), F/M, *p*NP, and finally, Y were determined. Sampling methods and examinations were all under guidance of the standard methods for examination of water and wastewater.

Changing conditions

While sludge age and injected *para*-nitrophenol concentration to the reactor were changing, at least 2 weeks (42 cycles) were considered for the system to be adopted with the new situation. It is assigned 3 days adaptation for changing influent COD, for determining kinetic coefficient (K_d, Y) in various sludge detention times (5, 10, 15, 20, 25 days), 1 week for changing sludge detention time (equal to 21 cycles), and 1 week for changing *p*NP concentration. All the measurements were carried out after stabilization; it is considered, and in normal condition (without adding *p*NP to the reactor), reactor operation was examined in various SRT (5, 10, 15, 20, 25 days). The SBR system worked very well without any bulking and foaming problems in 10 days retention time; so, it was selected as the optimum detention time, and all the experiments were carried out in this time. Maximum COD removal efficiency was achieved in SRT = 10 days without any bulking and foaming problems.

Concentration of suspended solids in the reactor and COD of effluent were considered as stabilization indicators. For more accuracy and certitude, all the samples were examined three times.

Results and discussions

Determination of *Y* and remaining COD in different amounts of injected *p*NP to the reactor

The rate of biomass changes per time, to COD changes per time are used for determining the biosynthetic coefficients especially *Y*. *Y* and sludge reduction in different concentrations of injected *p*NP to the reactor are shown in Table 1.

Table 1 Effect of injected *p*NP on *Y*, percentage of sludge reduction, and remaining *p*NP after reactions

Sludge reduction	*p*NP in effluent (mg/L)	*p*NP in sludge (mg/L)	$Y\,\frac{\text{mg Biomas}}{\text{mg COD}}$	*p*NP injection to the reactor (mg/L)
0	0	0	0.63	-
20	0.06	0.05	0.54	14.3
50	0.07	0.06	0.42	33.4
70	31	0.09	0.36	42.85
100	36.7	0.09	0.27	57.15
120	70	0.1	0.17	73
130	-	-	0.08	87.3
150	103	0.5	0	100

As it can be seen in Figure 2, in 10 days, sludge retention time (maximum efficiency of COD removal), *Y* per gr used COD is 0.63. By adding *p*NP to the reactor, biomass yield will be decreased, as in 100 mg/L and 130 mg/L of injected *p*NP, the coefficient of biomass production rate was calculated, respectively, as 0.27 and 0.08 gr produced biomass per 1 gr used COD. Many researchers achieved similar results with other uncoupling combinations in the reduction of produced excess biological sludge by decreasing biomass production coefficient [27,40,42]. The reason of decreasing sludge production coefficient was energy spilling because of uncoupled *para*-nitrophenol in the reactor as the result of less electron transportation from electron donor [42].

Figure 2 The effect of *p*NP injection on the biomass yield (*Y*), SRT = 10 days.

As it can be seen in Figure 3, removal efficiency of COD will be 95% without increasing *p*NP and with input COD of 600 mg/L, but *Y* reduced by adding *p*NP to the reactor, so that *Y* coefficient reached to 0.27 mg biomass/mg COD by adding 100 mg/L *p*NP to the reactor, which caused decreasing production of sludge. However, the problem was the increasing soluble COD in effluent by 193 mg/L (COD removal efficiency reached 75%). By increasing *p*NP to 130 mg/L, COD removal efficiency decreased by 56%, and effluent COD reached 360 mg/L. Table 2 shows results of the *p*NP chemical uncoupling compared with other researchers in minimization of biological excess sludge.

Figure 3 Effect of *p*NP ratio on COD removal efficiency, SRT = 10 days.

Table 2 Comparing *p*NP uncoupling results with other literature data in biomass minimization

Uncoupling chemicals and operating condition	Sludge reduction%	COD reduction%	References
35 mg/L of 2,4-Dinitrophenol to the continuously activated sludge reactor, *T* = 20°C, 0.3 g MLSS/g COD, pH = 7, MLSS = 2.5 g/L, SRT = 15 days, HRT = 5.5 h	0.3	Decreased by 3.7	[38]
2 to 2.5 mg/L 2,4,5-Trichlorophnol in continuously activated sludge culture, *T* = 20°C, pH = 7, MLSS = 2.5 g/L, SRT = 5 days, HRT = 3.5 h	50	-	[39]
0.8 to 1 mg/L 3,3,4,5-Tetrachlorosalicylanilide in continuously activated sludge reactor, *T* = 20°C, pH = 7, HRT = 8 h, SRT = 7 days, MLSS = 2 g/L	40	Nearly unaffected	[15,42]
Adding 20 mg/L of m-chlorophenol (mCP), batch-activated sludge culture, *T* = 25+1°C, pH = 7	86.9	Decreased by 13.5	[40]
Continuous addition of 100 mg/L *para*-nitrophenol in continuous mono culture of *P. putida*, *T* = 30°C, pH = 6.2/7.0	62 to 77	-	[28]
Continuous addition of 100 mg/L *p*NP, continuous activated sludge culture, dilution rate = 0.29/h, sludge discharge rate = 0.02 l/h, MLSS = 0.71 g/L, *T* = 20°C, pH = 7.7	49	Decreased by 25	[29]
Pilot plant scale, synthetic wastewater, application of TCS in conventional activated system at 1% g TCS/g MLSS	80	Nearly unaffected	[30]
Para-nitrophenol (*p*NP), sequencing batch reactor culture, *T* = 25°C, pH = 7.6, influent COD = 600 mg/L, addition *p*NP by:	-	COD in effluent (mg/L)	In current research
1. 50 mg/L	34	117	
2. 100 mg/L	57	165	
3. 120 mg/L	73	284	
4. 150 mg/L	100	480	

Effluent of various rates of injected *p*NP on SOUR and SVI

Figures 4 and 5 show that without *p*NP injection and addition of 100 and 130 mg/L *p*NP, SOUR was calculated with results 19, 31, and 7 mg used oxygen per h per gr MLVSS and SVI was 95, 48, and 13 mL/gr, respectively. It appears from Figure 3 that by adding *p*NP in the reactor, microorganisms have more activity up to a certain range, and consequently, SOUR rate increases up to 70 mg/L of *p*NP and decreases in more concentration of *p*NP because of its toxicity.

Figure 4 The effect of *p*NP injection on SOUR, SRT = 10 days.

Figure 5 The effect of *p*NP injection on SVI concentration, SRT = 10 days.

Mayhew and Stephenson in 1998 also reported the similar result by adding 2,4-dinitrophenol. They found out that uncoupled compounds caused break downs and energy loss in the culture; thus, SOUR increasing is not related to the growth [15]. Chen in 2002 found that in the presence of 0.8 mg/L TCS uncoupled compounds, SOUR increases 50% in the reactor, and increasing microbial activity happened in the presence of TCS in operating duration. High oxygen consumption indicates energy loss for metabolic conduction which ends to sludge minimization [43]. With increasing *p*NP concentration, as the result of toxicity of these metabolism uncoupling combinations on microorganism, SOUR will be reduced and so most of the microorganisms will get deactivated (except for few capsular ones). Microbial activity will be reduced and SOUR (breath rate) as a proper indicator, shows microbial death. SOUR reduction shows reduction of microorganism as the result of *p*NP presence in the system.

As it can be seen in Figure 5, SVI reduced by increasing injected *p*NP which caused low biomass production coefficient. When injecting *p*NP was increased more than 100 mg/L to the reactor, SVI reached to 48 mL/gr, and when *p*NP was increased to 130 mg/L, SVI reached 13 mL/gr, but in 150 mg/L of *p*NP injection, no sludge was produced.

BOD_5 and TSS in the effluent with various *p*NP injections

Figure 6 shows BOD_5 and TSS in effluent when different *p*NP concentrations are injected to the reactor. As it can be seen in the reactor, by increasing *p*NP up to 100 mg/L, BOD_5 and TSS of the effluent increased. When no *p*NP was injected, BOD_5 and TSS in the effluent were 18 and 28 mg/L, respectively. However, with the 100 mg/L *p*NP adding to the reactor, effluent BOD_5 and TSS will be 96 and 112 mg/L, respectively. Also, by adding 150 mg/L *p*NP to the reactor, effluent BOD_5 and TSS will be 17 and 230 mg/L, respectively. The cause of such a BOD_5 reduction is microorganism death as the result of *p*NP increasing concentration.

Figure 6 BOD_5 and TSS in the effluent in various *p*NP concentrations.

Comparing effluent COD with disposal and reuse standards in different *p*NP injection

As it can be seen in Figure 7, in 10 days cell retention time (maximum COD removal efficiency) only *p*NP concentration lower than 20 mg/L can be accepted by receptive water environmental rules, but effluent standards used in agricultural works, in *p*NP dosages higher than 100 mg/L, cannot estimate standards of irrigation and agriculture.

Figure 7 The comparison of effluent COD in the reactor with standards in various *p*NP injection.

Conclusion

Metabolic uncoupling is effective in reducing sludge production from the activated sludge process. In the 10 days of cellular retention, time of *Y* was 0.63 per gr used COD, but this amount was 0.27 and 0.08 gr biomass/gr COD if injected *p*NP increases to 100 and 130 mg/L. COD removal efficiency was 95% if the influent COD was 600 mg L-1, without *p*NP injection. However, biomass yield reduced by injecting *p*NP to the reactor: *Y* reached to 0.27

mg biomass/mg COD by injecting 100 mg/L *p*NP. This fact may cause sludge minimization, but its problem was a small increase in effluent COD which may be about 193 mg/L (COD removal efficiency will be 75%). SOUR amounts were 19, 31, and 7 mg used oxygen per h per gr of MLVSS, when amounts of injected *p*NP were 0, 100, and 130 mg/L, respectively. It means that in high concentrations, *p*NP behaves like a bacterial growth limiter. SVI rate reduced by the increase of injected *p*NP because biomass production reduced then. SVI was 48 mg/L when the injected *p*NP was more than 100 mg/L, and it was 13 mg/L if *p*NP was 130 mg/L, but there was no sludge made if injected *p*NP amount was 150 mg/L. Also, sludge minimization happened by increasing degradation resistant compounds such as *para*-nitrophenol, but it is necessary to reduce pollutant concentration in the effluent to an acceptable environmental standard amount.

Abbreviations

SBR, Sequencing batch reactor; COD, Chemical oxygen demand; BOD, Biochemical oxygen demand; SVI, Sludge volume index; SOUR, Specific oxygen uptake rate; VSS, Volatile suspended solids; *Y*, Biomass yield; SRT, Sludge retention time

Competing interests

The authors declare that they have no competing interests.

Authors' contributions

AT designed and conceived of the study; generated, assembled, analyzed, and interpreted the data; and drafted and revised the manuscript. AE designed and conceived of the study; collected, assembled, and interpreted the data; and drafted the manuscript. Both authors read and approved the final manuscript.

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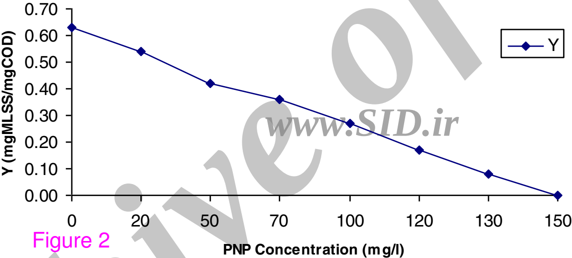
Figure 1


0.70
0.60
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Y (mgMLSS/mgCOD)
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130
150
PNP Concentration (mg/l)

Figure 2

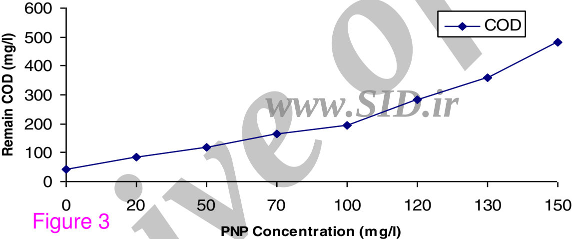
COD
Remain COD (mg/l)
600
500
400
300
200
100
0
0
20
50
70
100
120
130
150
PNP Concentration (mg/l)

Figure 3

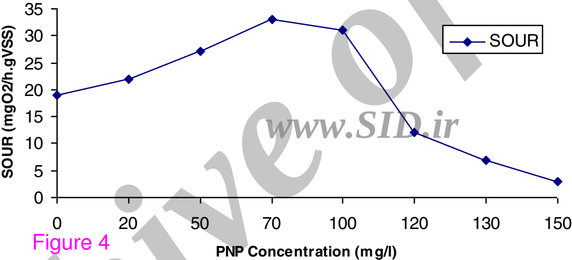
SOUR
SOUR (mgO2/h.gVSS)
0
5
10
15
20
25
30
35
0
20
50
70
100
120
130
150
PNP Concentration (mg/l)

Figure 4

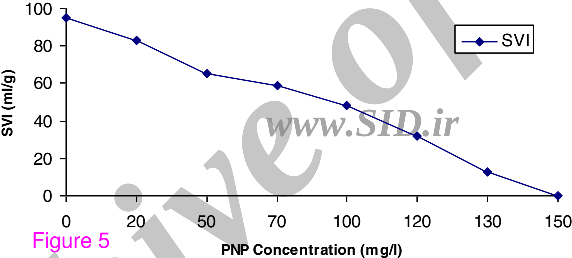
SVI
SVI (ml/g)
100
80
60
40
20
0
0
20
50
70
100
120
130
150
PNP Concentration (mg/l)

Figure 5

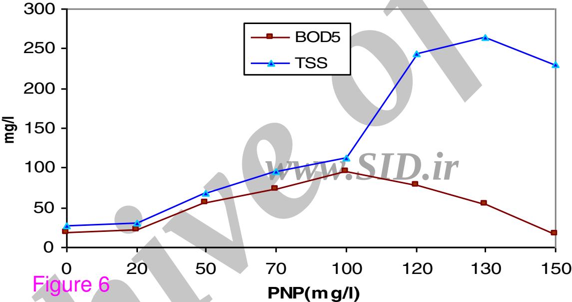
300
250
200
150
100
50
0
mg/l
BOD5
TSS
0
20
50
70
100
120
130
150
PNP(m g/l)

Figure 6

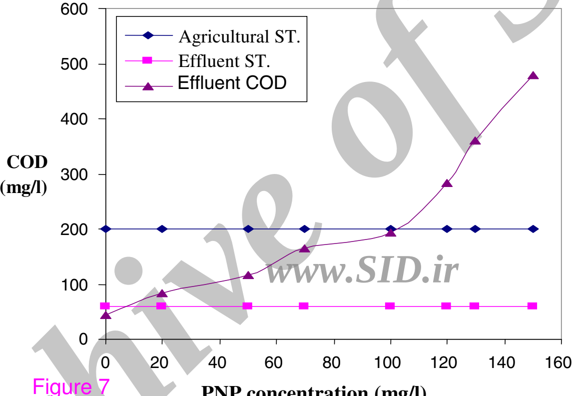
Agricultural ST.
Effluent ST.
Effluent COD
COD (mg/l)
0
100
200
300
400
500
600
0
20
40
60
80
100
120
140
160
PNP concentration (mg/l)

Figure 7