



Effect of n-hexane extracted from food wastewater on biological substrate adsorption in wastewater treatment

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ABSTRACT

The purpose of this study was to assess the effects of n-hexane contained in food wastewater on biological substrate adsorption (substrate bio-sorption). To achieve the purpose, microbial activity was assessed based on substrate removal characteristics and specific oxygen uptake rates (SOUR) at different concentrations of injected n-hexane. The X-ray photoelectron spectroscopy (XPS) was applied for an overall observation of n-hexane effects on substrate bio-sorption. The result showed that with higher n-hexane levels, the TBOD₅ concentrations increased in the effluent, thereby pushing down substrate removal efficiencies. The SOUR values fell from its maximum 86.4–38.6 mg O₂/g MLVSS h, which indicates that microbial activity was affected by n-hexane injection. In addition, the sludge injected with n-hexane was analyzed by XPS, and it was found that carbon elements (especially C–C and C–H) were gradually reduced on the sludge surface. The injection of n-hexane is assumed to inhibit microbial substrate adsorption, consequently reducing extracellular polymeric substances. Therefore, n-hexane needs to be removed sufficiently through a pre-treatment process in food waste-to-resource facilities.

Keywords: Food wastewater; n-hexane; Bio-sorption; Specific oxygen uptake rate (SOUR); X-ray photoelectron spectroscopy (XPS)

1. Introduction

The number of facilities for food waste treatment and recycling has steadily been growing since food waste landfill was banned. Such expansion of waste-to-resource facilities, which is attributable to the increasing efforts for recycling food waste, has resulted in an annually rising high-concentration organic wastewater from the cleaning and dewatering processes for desalination in those facilities. The

currently applied methods for treating water from food waste include ocean discharge and inland treatment, although the former is mostly preferred to the latter for economic reasons.

International agreements such as the London Dumping Convention and its 1996 Protocol, however, have already been imposed on land waste discharge into the oceans. In addition, there is a growing demand for regulations from fishermen who allegedly suffered from ocean dumping. In these circumstances, controls on waste disposal at sea have steadily been tightened. Thus, food wastewater produced by food

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waste-to-resource facilities needs to be treated inland. Food wastewater contains high-concentration salts, recalcitrant organic matters, and n-hexane that could affect biological treatment. This leads publically-owned treatment works (POTWs) to avoid a combined treatment of food wastewater. When flowing into biological treatment facilities, in particular, n-hexane tends to be adsorbed onto the surfaces of microorganisms, thereby inhibiting substrate bio-sorption and reducing biological removal efficiencies of organic matters.

Bio-sorption means the activity of organics in wastewater moving to activated sludge. It involves a series of consecutive stages: Retention in the floc [1], colloid hydrolysis by enzymes released from cells, and assimilation by bacteria [2,3]. Bio-sorption generally occurs as a result of electrostatic and hydrophobic activities according to the properties of organics [4]. There are still no sufficient studies on biological adsorption models which can explain the mechanism related to the behavior of organics [5]. Adsorption is a physicochemically removing process and directly linked with sludge age and concentration, soluble organics' chemical properties, etc. Adsorption by activated sludge, which is also known as biological adsorption, is reported as a mechanism which would be finished within 30 min when activated sludge contacts wastewater. Bio-sorption is related to microbial metabolism, biological availability of substrates, solid loading, and contact time [6].

This study aims to assess the effect of n-hexane contained in food wastewater upon biological processes. The effect of n-hexane in food wastewater upon microbes is investigated in association with biological adsorption, based on the following assumptions: (1) Substrates will be adsorbed to extracellular polymeric substances (EPSs) that are created on the surface of microorganisms; (2) n-hexane in food wastewater will be adsorbed to EPSs, thereby inhibiting substrate adsorption; (3) this will reduce organic treatment efficiency; and (4) subsequently slow down microbial activity. To this end, the membrane biological reactor (MBR) was chosen among the biological processes, and a laboratory-scale reactor was built and operated. Characteristics of substrate (TBOD₅) removal were derived at different concentrations of injected n-hexane. At the same time, the specific oxygen uptake rate (SOUR), which is generally used as an indirect indicator of microbial activity, was applied to observe how microbial activity would change with n-hexane injection into the reactor. In addition, elemental contents adsorbed to the sludge surface were analyzed using the X-ray photoelectron spectroscopy (XPS) to assess the effect of injected n-hexane on substrate bio-sorption.

2. Materials and methods

2.1. Equipment for experiments

A laboratory-scale reactor was designed to consist of three stages: stage 1 (shift reactor, Anoxic → Oxic, 0.15 × 0.25 × 0.41 m length, width, height), stage 2 (shift reactor, Anoxic → Oxic, 0.16 × 0.25 × 0.40 m), and the submerged membrane filtration stage (0.09 × 0.25 × 0.38 m). Fig. 1(a) and (b) show a diagram of the MBR, and its process used in this study. The reason for applying alternative reactions in this study is to treat nitrate nitrogen (NO₃⁻-N) and ammoniacal nitrogen (NH₃-N) simultaneously by adjusting reaction time. In addition, submerged flat-sheet membranes were applied after the biological treatment stages were completed. The membranes were made of polyethylene terephthalate. The spacer and reinforcing frame were made of polypropylene and polyester, PVC and ABS, respectively (Table 1). The pore size was 0.40 μm. The effective filtration area of each flat-sheet membrane was 0.05 m². The membrane flux was 7.34 L/m²h per membrane (3 membranes applied in total, thus 22.1 L/m²h). A constant membrane flux was maintained throughout the operation because this study is to investigate the effect upon biological treatment, not membrane fouling. The influent pump was controlled by a water level sensor to maintain a constant water level in the bioreactor. The membrane-filtered effluent was obtained by suction using a pump connected to the membrane. To assess the effect of n-hexane injection upon microbes, some conditions of the bioreactor were fixed as follows: pH (7–8), reaction temperature (25°C), flow (80 L/d), sludge retention time (SRT) (30 d), HRT (11.9 h), and membrane flux (22.05 L/m² h, 3 membranes). As seen in Fig. 1(c), the oxygen uptake rate (OUR) measuring instrument includes substrates, sludge, washing water input, an OUR measurement reactor, a dissolved oxygen (DO) measuring instrument, and a program that controls the whole system.

2.2. Effect of n-hexane injection on substrate removal

The influent used in this study was synthetic wastewater made up with CH₃OH, NH₄Cl, and KH₂PO₄ based on the average influent water quality of a POTW. The concentration of influent substrate (TBOD₅) was fixed at 130 mg/L using CH₃OH. As for the nitrogen concentration, NH₄Cl was used to make 30 mg/L of ammonia nitrogen (NH₃-N). In addition, 4 mg/L of PO₄³⁻-P was made using KH₂PO₄. After being extracted from food wastewater, n-hexane was injected at various levels (n-hexane conc.) according to experimental conditions. Conditions such as flow,

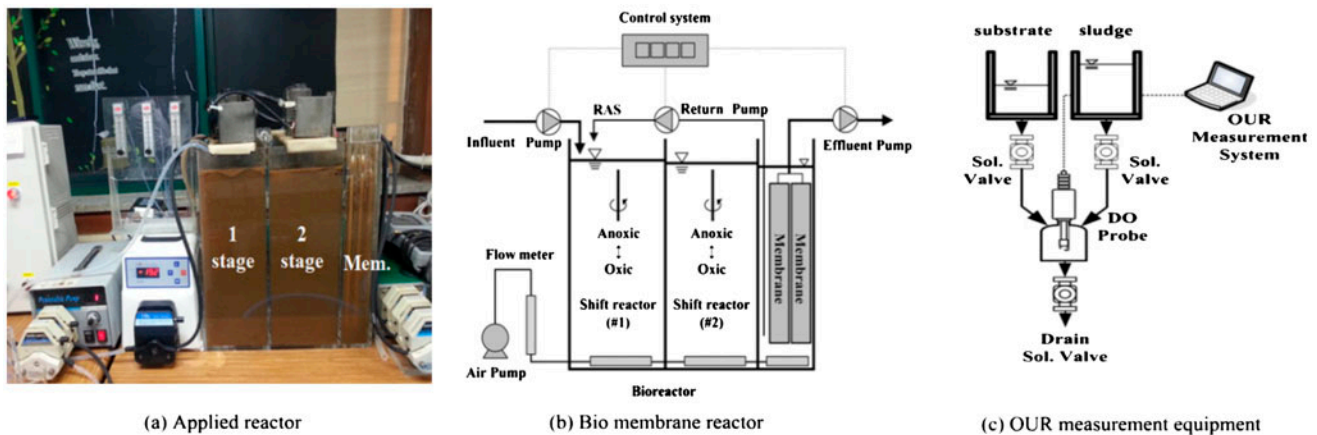


Fig. 1. Diagram of laboratory-scale MBR and OUR measurement reactor.

Table 1
Materials and specifications of membrane elements

Items	Specification
Effective membrane area, m ²	0.05
Nominal pore size, μm	0.25
Standard flux, L/ m ² h	12.5–20.8
Membrane	Polyethylene terephthalate
Spacer	Polypropylene and polyester
Reinforcing frame	PVC and ABS
Operation temperature, °C	2–38
Operation pressure, Pa	-4.9×10^4
Applicable pH range	3–10

SRT, DO concentration, pH, and temperature were consistently maintained. The operational settings of the reactor are shown in Table 2.

n-hexane was directly injected into the influent on stage 1 of the reactor. Continuous input was performed in accordance with the concentrations set for each mode. All analyses were initiated after 10 d of the operation in each mode. The operation was carried out for 30 d in each mode (the operation period: January–October 2013).

To derive substrate removal characteristics along with varying n-hexane concentrations in the reactor, experiments were carried out at different n-hexane

levels. The operational condition for each mode is seen in Table 3.

2.3. SOUR measurements at varying n-hexane levels

To analyze indirect effects of various n-hexane concentrations on microorganisms, SOUR were measured with the OUR instrument. Fig. 2 describes how to draw OUR from information sent by the OUR instrument [7]. The DO concentrations were recorded in a computerized system on a real-time basis from the measuring instrument (Fig. 2(a)). After valid, values

Table 2
Experimental conditions of MBR

Q (L/d)	V (L)			HRT (h)			SRT (d)	Membrane LMH (L/m ² h)
	Stage 1	Stage 2	Membrane	Stage 1	Stage 2	Membrane		
80	15	15.6	8.6	4.6	4.8	2.5	30	22.1

Table 3
Experimental conditions with concentrations of n-hexane

Mode (n-hexane injection concentration, mg/L)	n-hexane
1	0
2	1
3	2
4	4
5	6
6	8
7	10
8	12

were collected from the measured DO concentrations (Fig. 2(b)), OUR were finally calculated through regression analysis (Fig. 2(c)).

2.4. Effect of n-hexane injection on substrate bio-sorption

To assess fluctuations in EPSs on the microbial surface at different n-hexane injection levels, XPS analysis was conducted. The analytical procedure were as follows: First, the sludge was sampled in various modes divided by n-hexane concentration. The sludge samples were then completely dewatered. Finally, the sludge surfaces were analyzed with an XPS instrument (ESCALAB 210).

2.5. Methods for analyzing water quality

Sampling and analysis were done at the same hour every day. Measurement items for the samples were analyzed in accordance with the Standard Methods (APHA, 2005). For the XPS, ESCALAB 210 made by VG Scientific was used, and Al Ka monochromatic (1,486.6 eV) was employed as an excitation source. The sludge samples were completely dewatered after

being dried at about 105°C for 24 h and analyzed without sputtering and etching their surfaces, in order to maintain the vacuum level of the XPS instrument at 10–12 mm Hg. Elements such as O1s and C1s contained in the samples were measured with a wide scanning spectrum to identify the binding energy and intensity.

3. Results and discussion

3.1. Characteristics of the influent

Characteristics of the influent observed during the operation are shown in Table 4. The operation was conducted with the same raw water and different n-hexane levels.

3.2. Assessment of biological removal of substrates at various levels of n-hexane

Table 5 shows TBOD₅ removal characteristics at different levels of n-hexane injection in the biological reactor. The correlations derived from regression analysis are seen in Fig. 3. When the n-hexane concentrations were 0, 1, 2, 4, 6, 8, 10, and 12 mg/L, the average TBOD₅ values in the influent were, respectively, 132.0, 132.4, 131.4, 133.6, 131.0, 130.8, 129.0, and 128.8 mg/L, and the average TBOD₅ values in the effluent were each 3.2, 3.6, 4.0, 4.2, 4.8, 9.6, 17.2, and 22.6 mg/L. Thus, the removal efficiencies averaged 97.6, 97.3, 97.0, 96.9, 96.3, 92.7, 86.7, and 82.5%, respectively.

This indicates that TBOD₅ removal efficiencies consistently dropped at higher n-hexane concentrations. When n-hexane was injected at 12 mg/L, the effluent TBOD₅ level was 22.6 mg/L, where in turn the removal efficiency was 82.5%. Compared to Mode 1 (0 mg/L, control), n-hexane was found to affect

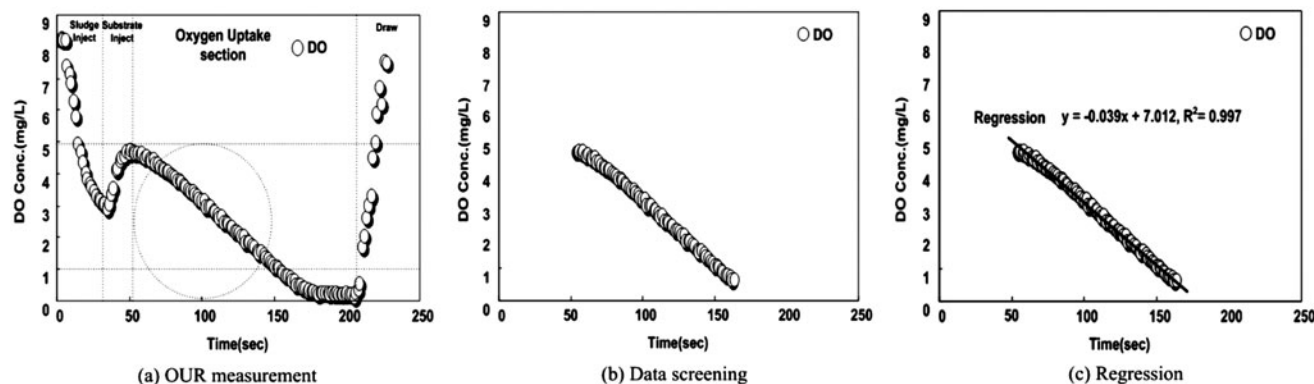


Fig. 2. Measurement of OUR, data screening, and data regression.

Table 4
Characteristics of influent in the laboratory-scale reactor

Constituent	Influent concentrations, mg/L		
	Min.	Max.	Ave.
TBOD ₅	127.1	133.7	131.1
NH ₃ -N	28.0	33.2	30.8
PO ₄ ³⁻ -P	3.7	4.3	4.0
Alkalinity	288.0	346.0	312.0

biological removal of substrates. A quadric function, $f(x) = 3.965 - 0.873x + 0.205x^2$ ($R^2 = 0.984$), was drawn from the correlations between n-hexane and the effluent TBOD₅ which were obtained through regression analysis.

Lee et al. [8] assessed organic destruction efficiencies in association with heavy metals. When Pb, Cd, and Cr were injected into the activated carbon-sequencing batch reactor process, the organic destruction efficiencies were 26.1, 36.4, and 43.8%, respectively. This indicates that the efficiencies clearly declined, compared with when the conventional sequencing batch reactor alone was operated, where the efficiencies were each 50, 46.4, and 54.5%. It is reported that the reason may be because heavy metals were adsorbed to sludge more quickly than organics or heavy metal ions were used as an inhibitor during the microbial metabolism. Shin et al. [9] increasingly added acid fermentation liquid from food waste to the biological reactor step by step. When the added amount became five times the theoretical demand, the concentration of SCOD_{Cr} in the effluent went up to 17 mg/L. This surge in the SCOD_{Cr} level is reported to be attributable to some of the organic matters in acid fermentation liquid that failed to be used by

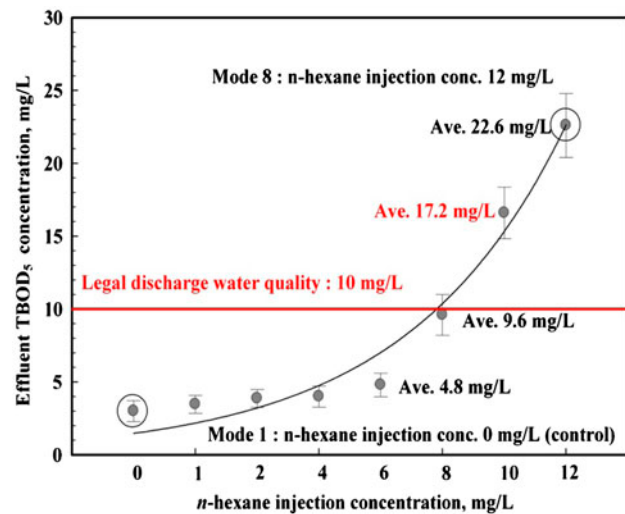


Fig. 3. TBOD₅ effluent concentration with injection of n-hexane.

microorganisms, which subsequently drove up the concentration of SCOD_{Cr} in the effluent.

Kim et al. [10] assessed the effect of salts on substrate removal. The COD_{Mn} removal efficiencies were analyzed after a phased increase in salt injection from 3,000 to 10,000 mg/L. At the salt concentration of 8,000 mg/L or less, the effect was so insignificant that the removal efficiency remained 91% or higher. At the concentration of 10,000 mg/L, however, the organic removal efficiency in normal conditions sharply falls to 87%. Ong et al. [11] studied TOC removal characteristics along with different nickel injection levels. When nickel was injected at 10 mg/L or higher, the removal efficiency falls below 88%. Compared with no nickel injection, more than 10% decrease in removal

Table 5
Substrate removal efficiencies in the laboratory-scale reactor

Mode	n-hexane injection conc.	Substrate conc. (mg/L) and removal efficiencies (%)								
		Influent			Effluent			Removal efficiencies		
		Min.	Max.	Ave.	Min.	Max.	Ave.	Min.	Max.	Ave.
Mode 1	0, mg/L	128.2	135.4	132.0	2.2	3.6	3.2	97.3	98.3	97.6
Mode 2	1.0	129.4	134.0	132.4	2.8	4.0	3.6	97.0	97.8	97.3
Mode 3	2.0	126.0	132.8	131.4	3.2	4.4	4.0	96.7	97.5	97.0
Mode 4	4.0	127.4	135.0	133.6	3.2	4.6	4.2	96.6	97.5	96.9
Mode 5	6.0	128.0	132.4	131.0	4.0	5.6	4.8	95.8	96.9	96.3
Mode 6	8.0	128.6	133.8	130.8	8.2	11.0	9.6	91.8	93.6	92.7
Mode 7	10.0	125.6	134.4	129.0	14.6	18.0	17.2	86.0	88.4	86.7
Mode 8	12.0	123.8	132.0	128.8	20.4	24.8	22.6	81.2	83.5	82.5

efficiency was reported. This study found that the TBOD₅ removal efficiency was 86.7% when n-hexane was injected at 10 mg/L. However, legal criteria for effluent water quality (under of 10 mg/L, Korea) failed to be satisfied. This indicates that n-hexane contained in food wastewater is one of the causes that inhibit substrate removal in the bioreactor.

3.3. Variations in microbial activity at different n-hexane levels

To explain the effect of injected n-hexane into the bioreactor on substrate removal in association with microbial activity, assessment was carried out based on SOUR measurements, which are used as an indirect indicator of microbial activity. The assessment results are shown in Table 6 and Fig. 4. When n-hexane was injected at 0, 1, 2, 4, 6, 8, 10, and 12 mg/L, the average SOUR values were 86.4, 85.8, 82.0, 80.7, 75.1, 62.8, 52.8, and 38.6 mg O₂/g MLVSS h, respectively. This indicates that the SOUR levels declined at higher n-hexane concentrations. With no n-hexane injection, the SOUR reached the highest (86.4 mg O₂/g MLVSS h). With n-hexane levels increasing, the SOUR values went down to as low as 38.6 mg O₂/g MLVSS h. Removal inhibition rates ranged from 0.8 to 55.3%.

Herrera et al. [12], who assessed the effect of fluoridation on microorganisms based on IC₅₀ (half maximal inhibitory concentration), reported that the inhibition rate reached up to 50% at the influent fluoride concentration of 148.8 mg/L. Han et al. [13] investigated how microorganisms were affected when PAC and Alum were directly injected into them. All microorganisms where coagulants were injected showed lower SOUR values than those without

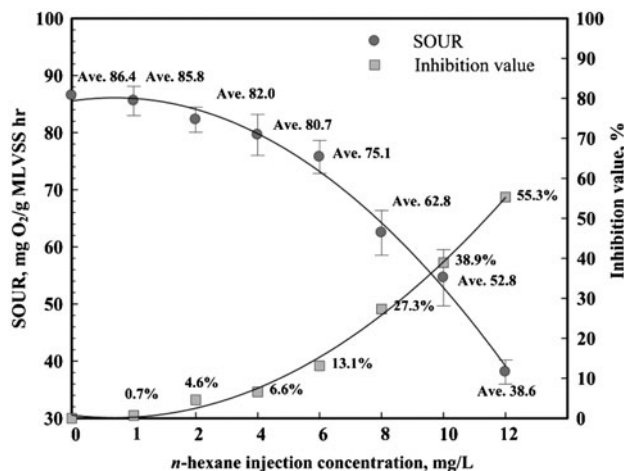


Fig. 4. SOUR and inhibition value with injection of n-hexane.

Table 7
At% of the C1s with n-hexane injection

n-hexane injection conc., mg/L	C1s At., %
0 (control)	64.95
1	62.45
2	61.22
4	61.08
6	57.62
8	55.25
10	31.80
12	23.65

coagulant injection. A study by Choi et al. [14], which was based on SOUR measurements to identify fluoride effect on substrate removal in association with

Table 6
SOUR data and inhibition value with injection of n-hexane

Mode	n-hexane injection conc.	SOUR ^a			Inhibition value ^b		
		Min.	Max.	Ave.	Min.	Max.	Ave.
Mode 1	0, mg/L	83.6	89.5	86.4	-	-	-
Mode 2	1.0	82.9	88.0	85.8	0.7	1.7	0.8
Mode 3	2.0	80.2	84.6	82.0	4.1	5.5	4.6
Mode 4	4.0	75.6	82.5	80.7	7.8	9.6	6.6
Mode 5	6.0	73.2	78.9	75.1	11.8	12.4	13.1
Mode 6	8.0	58.4	66.2	62.8	26.0	30.1	27.3
Mode 7	10.0	49.2	55.8	52.8	37.7	41.1	38.9
Mode 8	12.0	35.8	39.9	38.6	51.4	57.2	55.3

^aSOUR: mg O₂/g MLVSS h = dO₂/dt/MLSS.

^bInhibition value: 100-[100×(SOUR at the tested conc./SOUR of the control)], %.

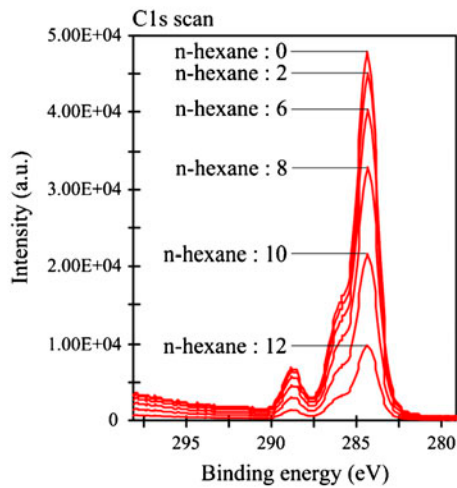


Fig. 5. The content of C1s with n-hexane injection.

microbial activity, also reported that when fluoride was injected at 0, 10, 50, 100, and 200 mg/L, the average SOUR values were 80.95, 54.75, 28.35, 20.68, and 16.54 mg O₂/g MLVSS h, respectively, which suggests that the higher the fluoride level was, the lower the SOUR was.

This study also observed decreasing SOUR and increasing TBOD₅ values in the effluent as n-hexane levels rose. Therefore, it is concluded that n-hexane injection into the biological reactor may affect microbial activity and n-hexane effect on microbial activity can be determined with SOUR measurements as an indirect indicator.

3.4. n-hexane effect on substrate bio-sorption measured by the XPS

The XPS was done to assess how n-hexane injection into the biological treatment process would affect

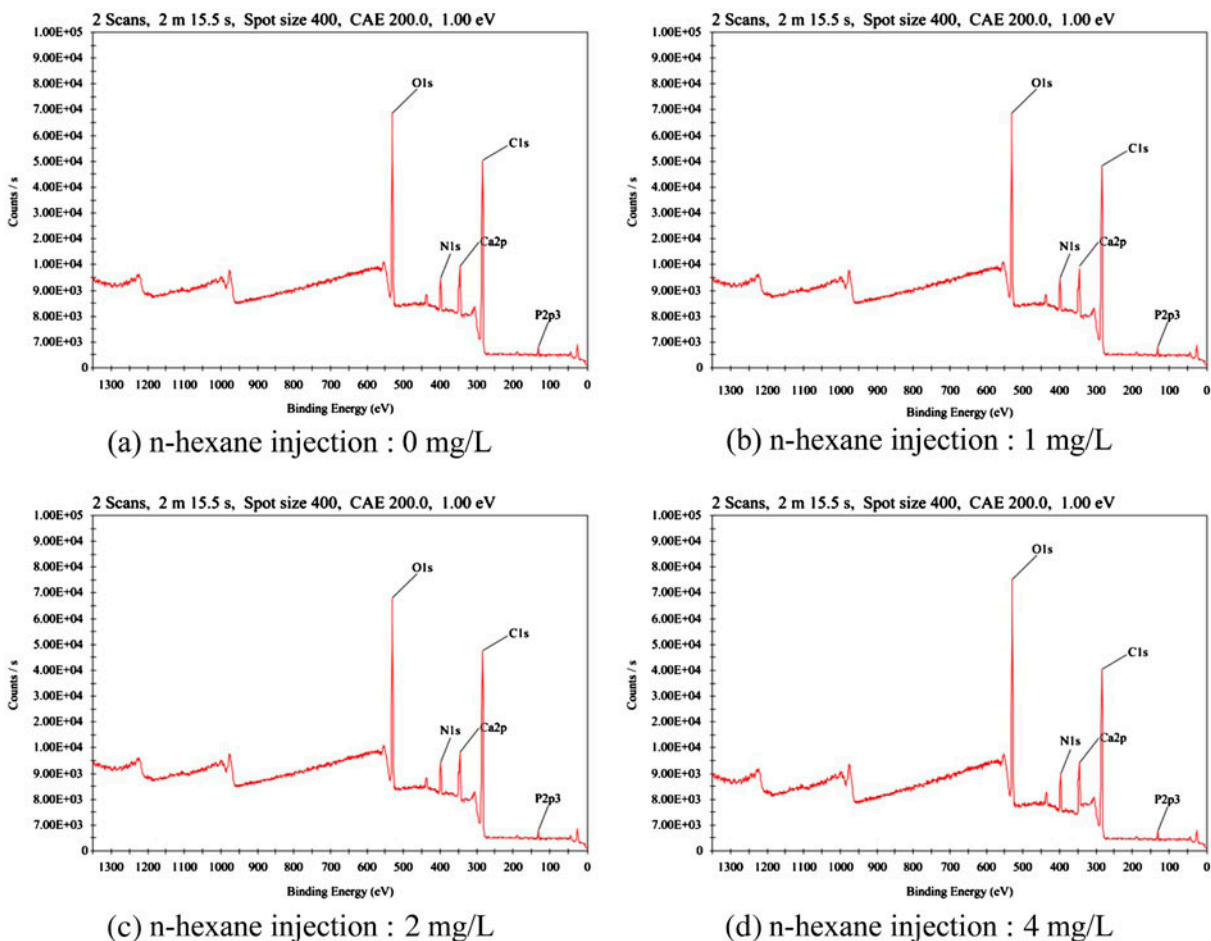


Fig. 6. XPS results with the n-hexane injection.

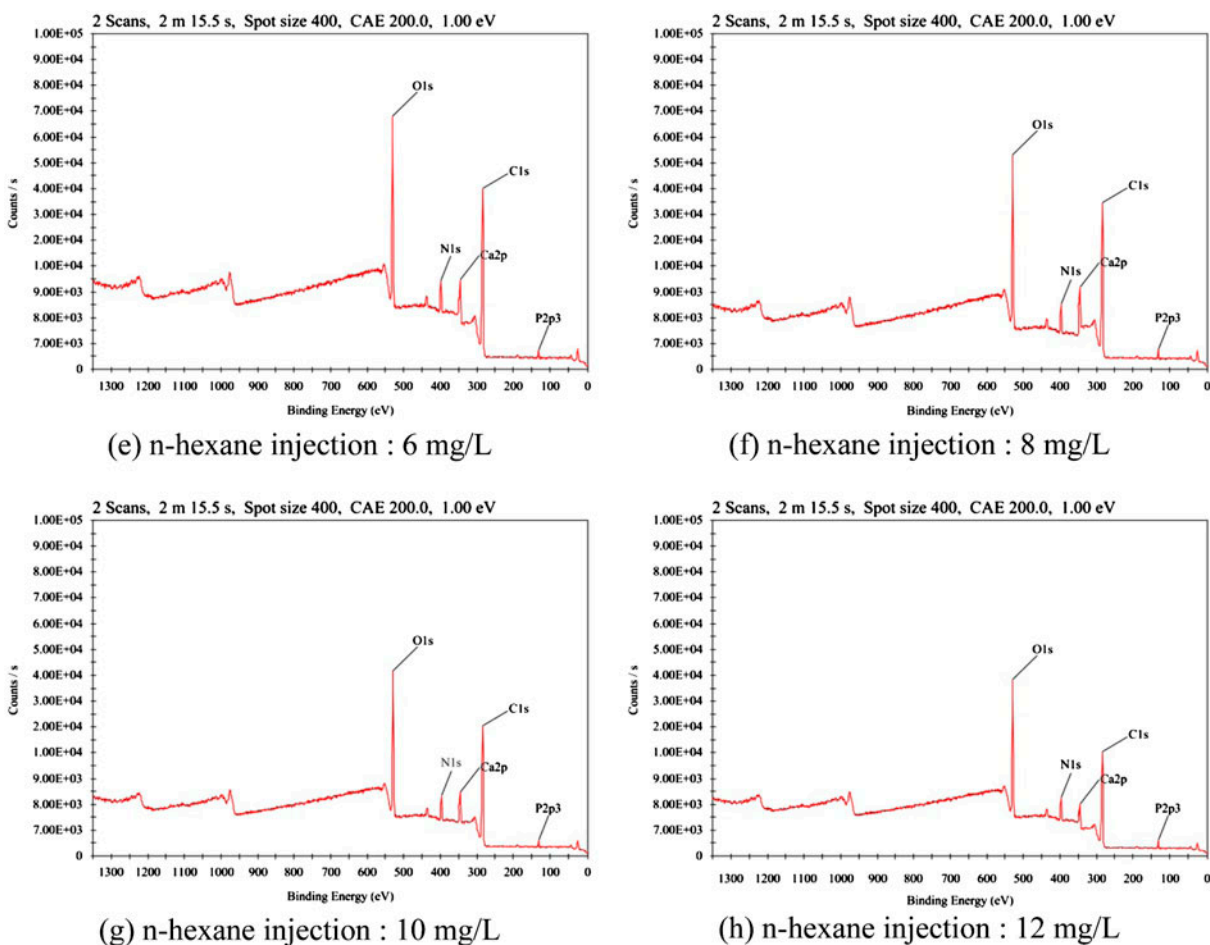


Fig. 6. (Continued).

biological substrate adsorption. The results are shown in Table 7, Figs. 5 and 6(a)–(h). Generally, XPS is used to measure atoms or elements adsorbed to the metal surface. In this study, XPS was employed to measure carbons adsorbed to the extracellular shell of the sludge. The electron shells of atoms are set by the octet rule and generally carry unique binding energy peak values. According to XPS measurement, the peak values of C1s and O1s were 284.6 and 532.5, respectively. Based on the existing literature, this study found that electron shells show the 1s orbital [15–18]. The sludge samples in each mode were completely dewatered, and their surfaces were analyzed with the XPS instrument to measure C element contents. The result showed that C element contents dropped along with increasing n-hexane levels: when n-hexane were injected at 0, 1, 2, 4, 6, 8, 10, and 12 mg/L, C element contents were each 64.95, 62.45, 61.22, 61.08, 57.62, 55.25, 31.80, and 23.65%. Correlations between C element contents on the sludge surface and n-hexane

concentrations were analyzed in association with TBOD₅ removal efficiency and microbial activity. The result indicated that n-hexane was adsorbed to sludge surfaces, thereby inhibiting substrate bio-sorption.

Volesky [19] presents that particle size and structure are important among the factors that affect bio-sorption. The same study also suggests that particle types and characteristics such as organics, inorganics, dissolved organics, particulate organics, and colloidal matters may have effect on biological absorption. As a result of reviewing studies that assessed SRT effect on biological adsorption, Kim et al. [20] report that there is a stage of organic adsorption prior to organic destruction by microorganisms in the biological reactor, which affects the whole organic removal efficiency, and that the amount of adsorbed SCOD per unit microorganism gradually decreases at increasing SRT. A study by Lim et al. [21] to assess pH effect on heavy metal adsorption removal using isolated strain *Exophiala* sp. LH2 found that the removal rates of Cr,

Cu, Ni, and Pb were 97.58, 89.84, 89.60, and 99.22% at pH 7 and reported that the heavy metal adsorption removal efficiencies dropped at lower pH levels.

In general, sludge which has adapted to a biological adsorption process where heavy-loaded organics flow in is known to show higher activities than activated sludge in a typical process. In a study by Ryu et al. [22] on correlations between livestock wastewater SRT and biological adsorption, it is reported that more than 80% of the total solids and about 74% of the dissolved solids were removed within only 40 min under the SRT condition of 9 d or longer, suggesting that SRT has effect on biological adsorption.

The XPS wide scan of C1s showed that the elements adsorbed to the microbial surface were in the form of C–C and C–H. According to the existing research, most EPSs are in the structure of C–C or C–H [23]. This study also found that C–C and C–H substances decreased, which indicates that EPSs were reduced as a result of inhibited substrate adsorption by n-hexane. Ucisik and Henze [24] report that the lower microbial substrate uptake may be attributable to the adsorption of calcium carbonates (CaCO_3) to EPSs. Noutsopoulos et al. [25] also suggest that lipids (fats, oils, grease) contained in sewage promote biological bulking, inhibit oxygen transfer, and increase odorous matters and effluent organics. Similarly, it was assumed in this study that n-hexane contained in food wastewater is adsorbed to EPSs and inhibits substrate adsorption.

This was proved through XPS and SOUR analyses as follows: (1) n-hexane was adsorbed to EPSs that were formed on the outside of microorganisms, (2) inhibited substrate adsorption to the microbial surface, and (3) reduced organic removal efficiency and microbial activity. Through this procedure, it was identified that n-hexane has effect upon biological treatment.

4. Conclusions

This study was conducted to investigate the effect of n-hexane injection into the biological reactor on substrate removal, microbial activity, and microorganisms. To this end, substrate removal characteristics were analyzed at varying n-hexane concentrations. At the same time, microbial activity analysis and XPS were done based on SOUR measurements to assess the effect of n-hexane injection on substrate biosorption. As a result, the following conclusions were drawn:

- (1) The higher the concentration of injected n-hexane was, the lower the TBOD_5 removal

efficiency was observed in the effluent. This indicates that n-hexane contained in food wastewater may affect substrate removal in the bioreactor.

- (2) It was found that SOUR values decreased along with increasing levels of n-hexane injection. The SOUR measurements were used to identify how n-hexane affects microbial activity. Thus, the SOUR results followed by n-hexane injection may be used as an indicator that helps objectively identify the effect of n-hexane in food wastewater on microbial activity.
- (3) It was found that carbon contents were dropped at higher n-hexane concentrations. This may be because n-hexane was adsorbed to the sludge surface and inhibited substrate adsorption and uptake, thus reducing EPSs and lowering organic removal efficiency and microbial activity.
- (4) Even a small amount of n-hexane could affect microorganisms and inhibit biological substrate removal. Therefore, when POTWs perform a combined treatment of wastewater from food-to-resource facilities, n-hexane needs to be removed sufficiently during the pre-treatment of food waste to minimize its effect on biological treatment.
- (5) This study was carried out to assess the effect of n-hexane contained in food wastewater on biological substrate removal. Further studies need to be conducted on substances such as high-concentration salts and recalcitrant organic matters besides n-hexane.

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