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ACTIVATED SLUDGE INHIBITION CAPACITY INDEX

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Abstract - Toxic compounds in sewage or industrial wastewater may inhibit the biological activity of activated sludge impairing the treatment process. This paper evaluates the Inhibition Capacity Index (ICI) for the assessment of activated sludge in the presence of toxicants. In this study, activated sludge was obtained from industrial treatment plants and was also synthetically produced. Continuous respirometric measurements were carried out in a reactor, and the oxygen uptake rate profile obtained was used to evaluate the impact of inhibiting toxicants, such as dissolved copper, phenol, sodium alkylbenzene sulfonate and amoxicillin, on activated sludge. The results indicate that ICI is an efficient tool to quantify the intoxication capacity. The activated sludge from the pharmaceutical industry showed higher resistance than the sludge from other sources, since toxicants are widely discharged in the biological treatment system. The ICI range was from 58 to 81% when compared to the synthetic effluent with no toxic substances.
Keywords: Biological treatment; Respirometric tests; Inhibition; Oxygen uptake rate.

INTRODUCTION

The activated sludge process is the most widely used treatment system for the removal of organic pollutants from wastewater (Yao *et al*., 2010) and is the focus of the present paper. Activated sludge contains a variety of biological organisms, which usually includes bacteria, protozoa, and rotifers. Bacteria form the major microbial community in the activated sludge (Kraigher *et al*., 2008). Currently a large amount of aromatic contaminants such as benzenes, phenols and anilines enter into the drainage system. Those contaminants are toxic and are hardly degraded by activated sludge in a typical industrial wastewater treatment plant (WWTP) (Cai *et al*., 2010).

Liwarska-Bizukojc and Bizukojc (2007) identified the effects of 50 mg L^{-1} non-ionic surfactants and found alterations in floc morphology. Chen *et al*. (2008) studied the effects of phenol (hydroxybenzene) on activated sludge microorganisms and concluded that 2,4-dinitrophenol reduced the sludge yield at various Solids Retention Times (SRT).

Wastewater characteristics and activated sludge behaviour have been evaluated through different approaches (Cai *et al*., 2010), among which the respirometric method has recently gained more attention (Cokgor *et al*., 2007). The principle of this approach relies on the fact that the respiration of the activated sludge is inhibited in the presence of toxicants, and hence, activated sludge respirometry reduction is a direct indication of toxicity (Tzoris and Hall, 2006).

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Standardised respirometric tests have been established (Cokgor *et al*., 2007) based on measures of the oxygen uptake rate of the bacteria. When wastewater contains toxicants or inhibitors, the oxygen consumption rate of activated sludge decreases. Albek *et al*. (1997) studied the effects of nickel, and Kelly *et al*. (1997) evaluated the effects of heavy metals (Cu, Zn, Ni, and Cd) on the activated sludge system. The inhibition of the biological activities of the activated sludge has ecological and financial consequences (Tzoris and Hall, 2006).

Gikas (2008) studied the effects of nickel and cobalt on the activated sludge and concluded that, at low concentrations, these ions act as micronutrients and, at high concentrations, as respiration inhibitors.

The efficiency of degradation processes in an aerobic reactor is highly influenced by temperature (Vogelaar *et al*., 2002), dissolved oxygen (Wilén and Balmér, 1999), organic matter, nutrients and micronutrients (Pamukoglu and Kargi, 2007). These factors must also be considered for an adequate operation of wastewater treatment plants. The discharge of compounds or toxic substances may negatively influence the microbiota community, impairing the performance of wastewater treatment at the industrial level (Surerus, 2009).

The objective of this study was to understand the relationship between the OUR and toxicants added. The inhibition capacity index (ICI) was determined after exposing activated sludge to various toxicants, such as copper ion, phenol (hydroxybenzene), surfactant (Linear alkylbenzene sulfonates) and amoxicillin (CAS number: 26787-78-0), in order to understand the respiration changes in the sludge. Four sets of assays were carried out using sludge from different wastewater plants: a pharmaceutical industry, two types of food industries and sludge obtained from a bread and cake industry Activated Sludge Reactor, acclimated with synthetic solution.

MATERIALS AND METHODS

Description of Industrial Wastewater and Synthetic Effluent Operation

Activated sludge was cultivated in a reactor at constant temperature (20 °C) in accordance with laboratory conditions. The effluents and the acclimated activated sludge were obtained from three distinct industrial wastewater treatment plants:

(1) Pharmaceutical industry: the sample was taken from the effluent equalisation tank that receives the industrial effluents and sewage discharges. It contained antibiotic residues and other potentially toxic substances. Nutrients were not added in order to simulate the real conditions of the industry.

(2) Margarine and cracker industry where the process includes an equalisation tank and complete clarification process. This effluent contained 20 to 50 mg L^{-1} of oil and grease. Phosphorous was added in order to maintain the relation COD:N:P = 150:5:1.

(3) Bread and cake industry: this sample contained no toxic compounds and no detectable oil and grease. Phosphorous was added in order to maintain the relation COD:N:P = 150:5:1.

(4) The fourth set of assays was carried out using an acclimatised synthetic effluent as described by Papadimitriou *et al*. (2007) containing 833 mg L^{-1} glucose, 2000 mg L^{-1} sodium acetate, 100 mg L^{-1} NaCl, 50 mg L^{-1} $CaCl_2 \cdot 2H_2O$, 20 mg L^{-1} KCl, 600 mg L^{-1} NH_4Cl and 333 mg L^{-1} $K_2HPO_4 \cdot 3H_2O$.

Table 1 summarises the characteristics of the effluents and the sludge used in the study.

All the effluents were collected after primary treatment, i.e., after solids, sands and greases were removed and after primary sedimentation.

Reactor Setup and Operation

The activated sludge was cultivated in a continuous flow activated sludge reactor with a working volume of 12 L, a sludge settling tank of 1.5 L, and an operating temperature of 20°C, maintained in an acclimatised room. The influent flow rate of the continuous flow activated sludge was 8 - 10 L day^{-1} in order to maintain the F/M relation at 0.15 mg COD.mg SSV^{-1}.day^{-1}. Test conditions are described in Table 2. The continuous flow activated sludge was operated during 30 min, with 3 min of influent filling and 3 min of sludge withdrawal from the bottom of the sludge settling tank to the biological reactor conducted with an air-lift system. The effluent was removed from the top of the settling tank by gravitational flow. Figure 1 shows the bench scale experimental apparatus used in the study, consisting of a biological reactor, equalisation tank, settling tank, portable air compressor and timer.

Concentration of Toxic Compounds

The compounds: copper, phenol, sodium alkyl benzene sulfonate and amoxicillin, according to Gerard (2006), listed in Table 3, were selected to simulate the discharge of substances widely used in households as well as in industrial processes.

Table 1: Influents and sludge characteristics of the effluents and the sludge.

Characteristics	Source of activated sludge			
	Pharmaceutical	Food (margarines and crackers)	Food (breads and cakes)	Synthetic
Flow rate ($m^3\ d^{-1}$)	306	104	38	-
Biomass VSS (mg L^{-1})	2926	2157	1445	3867
Biomass TSS (mg L^{-1})	4241	2765	2095	4838
N (mg L^{-1})	15.7	9.2	45	154
P (mg L^{-1})	2.5	2.1	4.3	34.6
pH	7.86	7.02	7.78	8.43
COD (mg L^{-1})	560	694[1]	1989[1]	2450
BOD (mg L^{-1})	80	341	954	1921

[1] The results were obtained after chemical coagulation, flocculation and flotation.
[*] Laboratory scale

Table 2: Operational parameters of the bench-scale wastewater treatment plants.

Feeding	10 L day^{-1}
Residence time in the aerated tank	2 days
Dissolved oxygen in the aerated tank	8 – 9 mg L^{-1}
pH influent	7 – 8.5
Mean temperature	20 °C

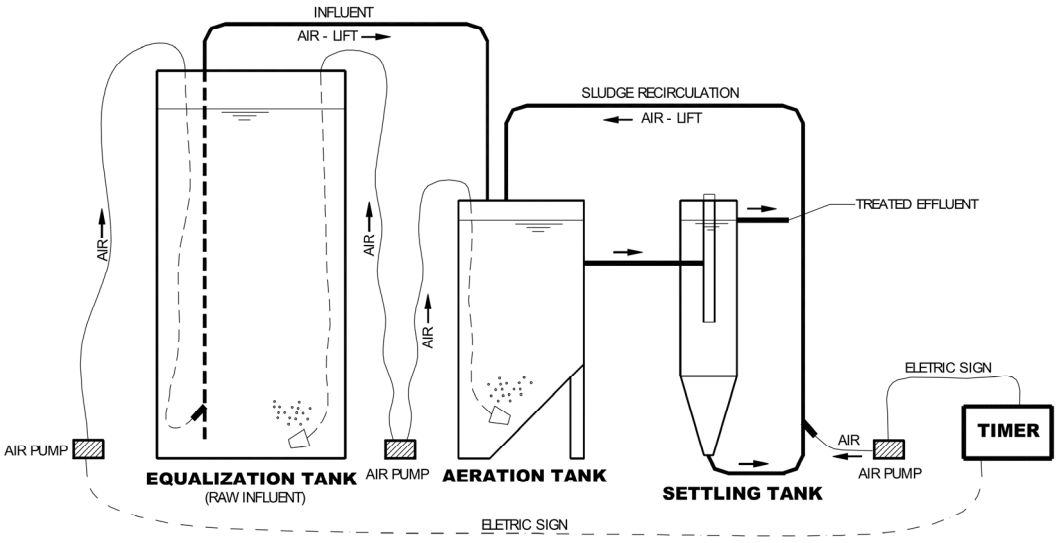

Figure 1: Experimental apparatus.

Table 3: Substances employed in the experiments.

Type of Compound	Active Principle	Concentrations (mg L^{-1})
Dissolved Copper[1]	Cu^{2+}	5 ; 10 ; 30
Phenol[1]	C_6H_5OH	50 ; 100 ; 500
Surfactant[*]	Sodium alkylbenzene sulfonate	17 ; 34 ; 85
Antibiotic[*]	Amoxicillin	4.5 ; 6 ; 8

[1] Gerard, 2006
[*] The concentrations were defined in the tests

Respirometric Batch Tests Procedure

The respirometric experiments were conducted in a 300 mL BOD bottle at a constant temperature of 20 °C. The sludge with or without one of the toxic compounds and/or substrate (sucrose) was added to the reactor, as described by Cokgor *et al*. (2007), to stimulate the readily biodegradable COD fraction and aerated for 15 min. Then dissolved oxygen (DO) was measured over 5 min and the Oxygen Uptake Rate (OUR) was calculated.

Respirometric tests were conducted using the procedure described in Figure 2, and the apparatus shown in Figure 3.

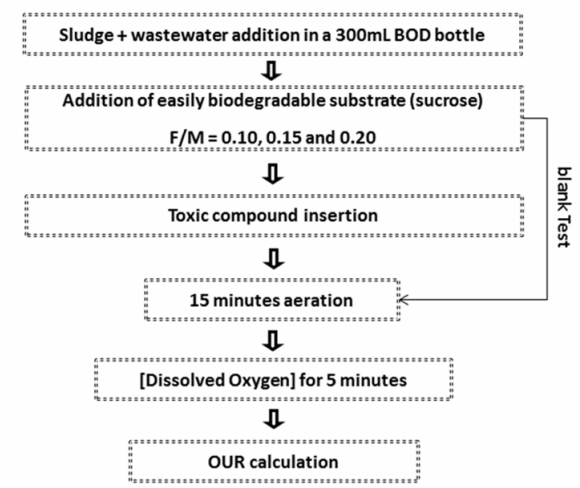

Figure 2: Experimental procedure for respirometric tests.

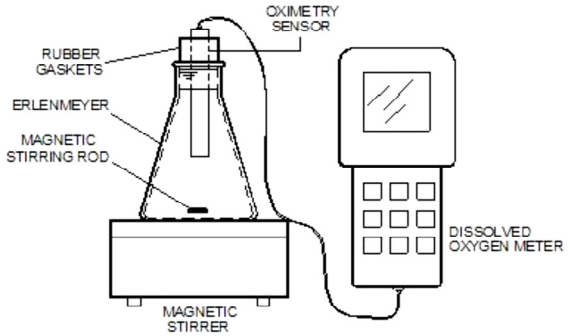

Figure 3: Equipments and apparatus for the respirometric tests

The calculation of F/M feeding the sludge for subsequent respirometric tests was conducted according to the equation:

$$F/M = \frac{DQO(mg/L)\cdot Q(L/dia)}{RNFT(mg/L)\cdot V(L)}$$

The solution was prepared with 30000 mg L^{-1} COD with sucrose, an easily biodegradable compound. The concentration of Volatile Suspended Solids (VSS) was measured for each sludge (Table 1) and the volume of sucrose solution calculated for each experiment was used to feed the BOD bottle.

Once the VSS (mg L^{-1}) was obtained (measured on the day of collection and acknowledged constant during all days of the experiment), the flow rate of substrate (sucrose) to be added to the 300 mL bottle to feed the sludge was calculated. Thus, using F/M = 0.15 and VSS of the pharmaceutical industry,

$$0.15 = \frac{30000(mg/L)\cdot Q(L/batch)}{2926(mg/L)\cdot 0.3(L)}$$

$$Q = 4.39\ mL/batch$$

To keep the conditions mentioned (F/M = 0.15), 4.39 mL of COD (= 30000 mg L^{-1}) are needed, as the example.

Inhibition Capacity Index (ICI)

Respiration tests were conducted after contaminating the sludge with the four chemicals under study. The inhibition capacity index of the chemicals tested at each concentration was calculated as:

$$ICI = C_{toxicant}\cdot SOUR\cdot t\cdot V$$

where $C_{toxicant}$ is the highest toxicant concentration used for a test, SOUR is the specific oxygen uptake rate, t is the contact time between toxicant and effluent/sludge, and V is the volume of effluent plus activated sludge.

The inhibition capacity index indicates the intoxication that the microorganisms can tolerate under sudden discharge of toxicant at high concentrations. The contact time between the toxicants and the microorganisms is an important parameter for the correct ICI calculation. In this study, we conducted experiments with an aeration time of 15 minutes.

RESULTS AND DISCUSSION

Respirometric Tests

Phenol concentrations as high as 500 mg L^{-1} did not considerably change the respiration rate of microorganisms from the pharmaceutical industry (Figure 4). This can be explained because of the high

microorganism selectivity of pharmaceutical industry sludge promoted by the usual presence of toxic compounds in the influent.

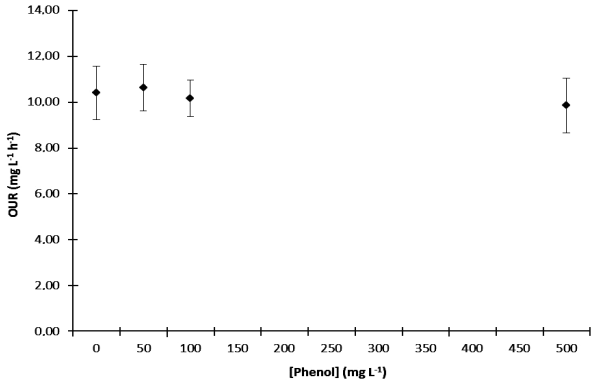

Figure 4: OUR x [Phenol]; pharmaceutical industry; temperature: 21.2 °C; F/M was kept at 0.15; the substrate added was sucrose; each point an graph represents the average of 3 assays and the standard deviation is indicated on the graph.

Amoxicillin is commonly employed in human prescription medicines as a therapeutic agent due to its broad spectrum against bacteria (Aksu and Tunc, 2005). However, in contact with activated sludge, amoxicillin is degraded by microorganisms as a substrate (Chen *et al*., 2011). The household discharge of this compound into the sewage does not cause activated sludge reactor interference due to the high selectivity of microorganisms present in the activated sludge. Figure 5 shows the relation between the OUR and amoxicillin in effluents from the margarine and cracker industry. Amoxicillin did not inhibit the microbial growth at relatively high concentrations.

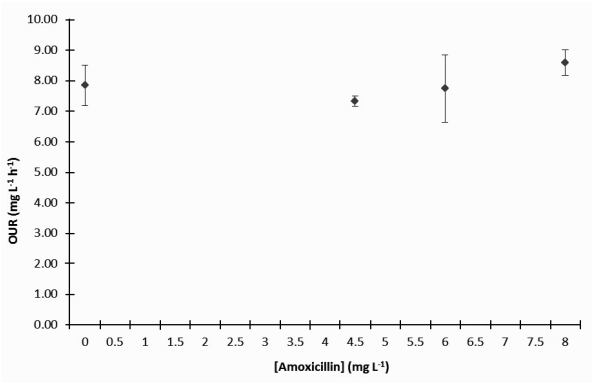

Figure 5: OUR x [Amoxicillin]; margarine and cracker food industry activated sludge; temperature: 22 °C; F/M was kept at 0.15; the substrate added was sucrose; each point on the graph represents the average of 3 assays and the standard deviation is indicated on the graph.

Heavy metal ions such as Cu^{2+} are known bactericidal agents (Lin *et al*., 1996) and may be used to validate the respirometric capacity of biomass. Figure 6 shows the effect of copper ions on the microorganisms and indicates an OUR decrease for the bread and cake industry activated sludge. Therefore, its intoxication impact seems to be greater.

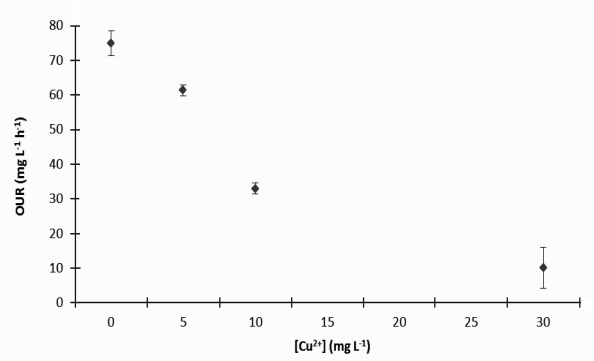

Figure 6: OUR x [Cu^{2+}]; bread and cake food industry activated sludge; temperature: 21 °C; F/M was kept at 0.15; the substrate added was sucrose; each point on the graph represents the average of 3 assays and the standard deviation is indicated on the graph.

Although oil and grease and toxic compounds were not present in the effluent, acclimated sludge from synthetic effluent suffered an OUR decrease with the increase in surfactant concentration (Figure 7). This behaviour may be explained by the absence of toxic compounds in the influent, thus decreasing the selectivity of microorganisms.

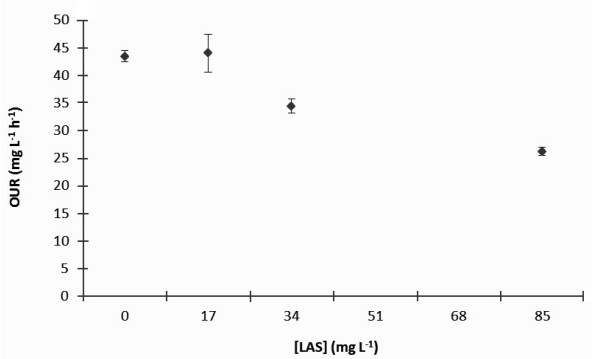

Figure 7: OUR x [LAS]; synthetic effluent; temperature: 20.2 °C; F/M was kept at 0.15; the substrate added was sucrose; each point on the graph represents the average of 3 assays and the standard deviation is indicated on the graph.

The behaviour of synthetic influent is different when compared to the wastewater from industries that contain toxic compounds, but it has an Oxygen Uptake Rate of the same order of magnitude as the effluent from the bread and cake industry, which does

not contain effluent toxicity. Based on the results of Table 5, the SOUR is greater than 1.5 for the synthetic wastewater and 1.9 for the bread and cake industry, indicating that these effluents are more susceptible to intoxication.

Therefore, the microorganisms from the industrial activated sludge have higher toxic compound resistance, leading to a lower oxygen consumption rate. All results are summarised in Table 4 and Table 5.

Table 4: Types of activated sludge used in the experiments with respective OUR values.

Type of activated sludge	OUR (mg L^{-1} h^{-1})
Margarine and crackers	9.70
Pharmaceutical	9.98
Bread and cakes	24.28
Synthetic	39.16

Table 5: SOUR applied to activated sludge obtained from distinct industrial wastewater treatment plants.

Activated sludge from distincts industries	Intoxicant compounds SOUR (mg DO mg VSS^{-1} h^{-1})			
	Cu^{2+}	Phenol	LAS	Amoxicillin
Pharmaceutical	0.04	0.06	0.06	0.04
Margarine and crackers	0.07	0.08	0.10	0.07
Bread and cakes	0.54	-	0.28	-
Synthetic	0.15	0.19	0.19	0.23

F/M = 0.15 applied along the test
DO = Dissolved oxygen
VSS = Volatile suspended solids

Inhibition Capacity Index (ICI) Example

The pharmaceutical sludge was intoxicated with 30 mg L^{-1} of Cu^{2+} to calculate the ICI. The average OUR value applied in the ICI example is indicated in Table 6:

$$ICI = C_{toxicant} \cdot SOUR \cdot t \cdot V$$

$$CI = 30\left(\frac{mg_{toxicant}}{L}\right) \cdot \frac{5.72\left(\frac{mg_{O_2}}{L.h}\right)}{4241\left(\frac{mg_{VSS}}{L}\right)} \cdot 15 \cdot \left(\frac{1}{60}h\right) \cdot 0.3(L)$$

$$ICI = 0.00303\left(\frac{mg_{toxicant} \cdot mg_{O_2}}{mg_{VSS}}\right)$$

$$< \ 0.01 \ (\text{quantification limit})$$

Toxicants decrease SOUR because they inhibit microbial activity. Therefore, higher resistance of activated sludge to toxicants may not significantly change the SOUR. Higher resistance of activated sludge to toxicants is related to a lower SOUR value at the same concentration of toxicant, as shown in Table 5.

Table 7 indicates the ICI values or all sludge and toxicants used in the experiments. The ICI and SOUR were observed to decrease with the increase in the microorganism's resistance, which is related to the effluent characteristics, as shown in Tables 5 and 7, because resistance of activated sludge may show lower SOUR for toxicant influent. This index can help in predicting the toxic effect of these contaminants on activated sludge at various concentration levels.

Table 6: Experimental results of the respiration test performed in triplicate.

$[Cu^{2+}]$ = 30 mg L^{-1} F/M = 0.15		$[Cu^{2+}]$ = 30 mg L^{-1} F/M = 0.15		$[Cu^{2+}]$ = 30 mg L^{-1} F/M = 0.15	
[DO] (mg L^{-1})		[DO] (mg L^{-1})		[DO] (mg L^{-1})	
8.40		8.35		8.77	
8.34		8.30		8.73	
8.29		8.25		8.67	
8.23		8.20		8.63	
8.17		8.15		8.60	
8.12		8.11		8.56	
8.06		8.06		8.52	
8.01		8.00		8.49	
7.95		7.95		8.46	
7.90		7.91		8.42	
7.84		7.86		8.39	
OUR	6.72	OUR	5.88	OUR	4.56

Table 7: Inhibition capacity index (ICI) for sludge from pharmaceutical, bread and cakes, margarine and cracker food industries and synthetic effluent intoxicated with Cu2+, phenol, amoxicillin and LAS.

Intoxicant	Inhibition Capacity Index ($mg_{toxicant}$ mg_{O2} mg_{VSS}^{-1})			
	Pharmaceutical	Food (margarine and cracker)	Food (bread and cake)	Synthetic
Cu^{2+}	< 0.01	< 0.01	0.01	< 0.01
Phenol	0.08	0.10	-	0.29
Amoxicillin	0.01	0.02	0.08	0.03
LAS	< 0.01	< 0.01	-	< 0.01

CONCLUSION

The inhibition capacity index (ICI) has proven to be useful to indicate the intoxication capacity that the activated sludge may suffer when compared with the ICI obtained from the synthetic effluent with no toxic substances. It may be attributed to microorganism resistance developed in effluents that continuously receive toxicants. In fact, the toxicants used in

this study that caused a respiration decrease may be used in the same concentrations in tests with other effluents.

The biomasses from synthetic wastewater and from the bread and cake industry were less resistant to toxic compounds, resulting in higher ICI because no intoxicating compounds were present in the influents.

The literature reports that experiments conducted under chronic intoxication reduce sludge production. The tests in this study were conducted under sudden intoxication and were validated because the sludge respirations showed similar behaviour.

The low ICI value (< 0.01) (Table 7) of the activated sludge suggests a delay in microorganism metabolism, which may decrease the biodegradation performance and hence cause a reduction in the process efficiency.

Low ICI values (<0.01) indicate susceptibly to intoxication by various compounds; on the other hand, high ICI values indicate that the industrial treatment plants are receiving toxicants, which should be avoided or reduced in the industrial process.

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