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RESEARCH ARTICLE

Remediation effects of adapted bacteria cultures on water quality: an example of petrochemical industry

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Abstract

In this study, it was aimed to make an efficient wastewater treatment by using adapted bacteria cultures to support and strengthen the biomass in aeration basin of the wastewater treatment plant, when the treatment processes are not sufficient in biological wastewater plants (BWWTP) for industrial wastewater which contains toxic and recalcitrant organic chemicals. For this purpose, petrochemical industry was chosen for field of study. The physical, chemical (flow rate, dissolved oxygen, temperature, chemical oxygen demand, total suspended solids, phenol, NH4-N, phosphorus, pH) and microbiological analysis (indicator microorganisms) were carried out predominantly to observe the performance of the treatment plants. Microbiologic data (during the applications) and chemical data which were recorded before and during the applications in the biological wastewater treatment plant of petrochemical industry, showed that adapted bacteria cultures could stabilize discharge limits from independent influent parameters. The results indicate that the adapted bacteria cultures easily induce healthy biomass during the biological treatment processes in the tested system. It can be suggested that this method may be applicable to develop healthy biomass in these types of wastewaters.

Keywords: wastewater microbiology, indicator protozoan, biological wastewater treatment

Introduction

Bioremediation, the use of microbial degradation processes to detoxify environmental contamination, was first applied to petroleum hydrocarbon contaminated ground water systems in the early 1970s. In the summer of 1971, a gasoline pipeline in Whitemarsh Township, Pennsylvania, broke and released several hundred thousand gallons of high – octane gasoline. Over the next year, an estimated 100,000 gallons (379,000L) of gasoline seeped to the underlying dolomitic aquifer and dissolved petroleum hydrocarbons migrated to a nearby municipal water supply well. At that time, it had only recently been proposed that ground water systems might contain microorganisms capable of degrading petroleum hydrocarbons (Litchfield and Clark 1973; Chapelle, 1998; Jamison *et* *al*. 1975). Since this beginning in the early 1970s, bioremediation of petroleum hydrocarbons has become an accepted technology and has grown into an industry making more than $290 million a year in the United States alone (Glass *et al*. 1995).

Using adapted bacteria cultures has become widespread during the recent years with the development in biotechnology. Adapted bacteria cultures do not replace naturally present bacteria in the aeration basins of biological wastewater treatment system; they only support them and bring synergy.

Each biological treatment process has its own profile of microorganisms when operating at a steady state condition. By seeing the wastewater microorganisms an operator is able to correlate the microorganisms with existing operational conditions. These conditions may be acceptable or not. Therefore, an operator can monitor the microorganism and determine whether operational conditions are acceptable or not, that is, use the microorganisms as indicators or "bioindicators" (Gerardi 2008).

The aim of this study is to prevent the negative effects on water quality in the discharge points with an efficient treatment on the industrial wastewater, which contains toxic and hazardous organic chemicals, by using adapted bacteria cultures to support and strengthen the biomass in the aeration basin of the wastewater treatment plant.

In this study, in order to research the effect for the removal rate of chemical and organic materials with using adapted bacteria cultures in the biological wastewater treatment systems, some applications were made in petrochemical industry and the results evaluated with chemical and microbiological analysis during the period between June 2008 to October 2008.

Materials and Methods

Study Area

Petrochemical industrial **-** biological wastewater treatment system was selected as the study area. Flow diagram of the system and characterization of wastewater are summarized in Table 1. Wastewater treatment plant is designed to make a treatment of 24,000 m^3/day chemical wastewater, 13,200 m^3/day oily wastewater, and 3,600 m^3/day domestic wastewater.

Intake of biological treatment plant in the petrochemical side of the samples taken at different times between 27.06.2008 and 27.10.2008 according to the results of the characterization of wastewater are shown in Table 1.

Adapted bacteria cultures are supplied from Novozymes Biologicals (France) company.

Table 1. Wastewater characterization of petrochemical industry.

Parameter	Value	Average
Flow	Domestic: 1,200 – 2,600 m^3/day	1,700 m^3/day
	Inlet Biological Treatment - Oily wastewaters (In outlet of Flotation): 10,000 – 16,000 m^3/day	12,000 m^3/day
Inlet COD	Domestic: 200 – 500 mg/L	250 mg/L
	Inlet Biological Treatment - Oily wastewaters (In Outlet of Flotation): 500 – 1500 mg/L	1000 mg/L
pH	Domestic 6.5 – 8.9	7.9
	Inlet Biological Treatment - Oily wastewaters (In Outlet of Flotation): 6.1 – 8.7	7.4

BI-CHEM® 1008CB encompasses a wide variety of organic degradation capabilities to target a variety of industrial wastes. The microbial blend incorporates strains capable of degrading solvents, fatty acids, hydrocarbons surfactants, ketones and recalcitrant organics. It improves waste system stability and reduces frequency and severity of upsets (Novozymes Biologicals, France).

BI-CHEM® 1002CG is a synergistic blend of selectively adapted bacteria for application to wastewater containing phenol, cresols and related aromatic compounds including catechol and cumene. Applicable processes include steel coking, coal conversion, petroleum cracking, plastic resins and pharmaceuticals. By using this type of adapted bacterial culture reduced the inhibitory effects of phenol by creating more resistant biomass to impact, against the toxic effects caused by phenol and so forth compounds (Novozymes Biologicals, France).

BI-CHEM® 1008 CB and 1002 CG adapted bacteria cultures are powdered products which consist of 25 pieces x 0,454 kg soluble packets. Total package weight for both is 11,35 kg. Those bacteria cultures in packets depending on the amount of daily dosage, thrown directly into the aeration tank once a day. Packets which contacted with wastewater, were immediately dissolved into the water to leave the active substance in them.

Dosage amounts were defined according to the daily wastewater flow rate (m^3/day) and inlet COD concentration to the biological treatment (m^3/L) (Novozymes Biologicals, France). Dosage program was prepared on the basis of study period flow rate (13,000m^3/day), domestic and inlet of biological treatment COD concentration (500 – 1,500 mg/L).

Physical and Chemical Analyses

Dissolved oxygen was recorded with the online measuring instrument (WTW, Zellweger Analytic SA). Flow rate was tested with flow-meter in the wastewater treatment plant (Ultraflux-Technoparc, Endress + Hauser). pH measurements were made using a portable kit (1.08027, Merck). Temperature values were recorded using automatic thermometer (Multical ®, Merck).

COD, Standard Methods 5220 B (open reflux method); TSS was done according to SM 2540 D (Eaton *et al*. 1995). Hach Lange test kits for the measurement of COD from time to time (150 - 1000 mg/L, LCK 114 and 1000 to 10,000 mg/L and COD LCK 014 kits) were used.

Phosphorus and nitrogen analysis were done according to TS 7924 EN 25663 and ASTM D 3590 methods (Eaton *et al*. 1995).

Microbiological Analyses

200 ml of the wastewater samples which were taken from the aeration tanks were transported under cold chain at +4°C to the Aquatic Microbial Ecology Laboratory, Faculty of Fisheries, Istanbul University and examined without delay.

Groups of protozoa microorganisms as amoeba, flagellates, free-swimming ciliates, crawling ciliates and stalked ciliates with rotifers, nematodes, algae and filamentous bacteria in the aeration tank were evaluated as indicator microorganisms with using Olympus BX51 Epi-fluorescence microscope (Gylmph 2005).

Results

In order to determine the status of the facility prior to the adapted bacteria culture application, intermittently input flow, pH, COD; dissolved oxygen in the aeration tanks and output COD, pH were measured at the biological treatment plant. In addition to the above analysis, mixed liquor suspended solid (MLSS) values in the aeration tanks were also measured in August 2008. Then, application of adapted bacteria culture has been started, at this time the same parameters were measured. The results of this study are shown in Tables 2.

The changes in MLSS concentrations are given in Table 4, according to selectively adapted bacterial culture application in the aeration tanks.

The range of COD discharge values further improved in October 2008 and became stable under 200 mg/L.

Table 2. Analysis values of wastewater treatment plant of petrochemical industry (June - July – August 2008 / Pre-application values).

	Inlet Biological Treatment						Aeration Tank A		Aeration Tank B		Outlet Biological Treatment			
	Flow		COD		pH		DO	Temp	DO	Temp	COD	ahl Nitro	Total - P	pH
Date	Domestic m^3/day	Outlet Flotation m^3/day	Domestic m^3/day	Outlet Flotation m^3/day	Domestic m^3/day	Outlet Flotation m^3/day	mg/L	T°C	mg/L	T°C	mg/L	mg/L	mg/L	
27.06.08	120	550	159	1838	7,6	9,5	1,68	27,90	1,55	28,32	1760			7,2
30.06.08	120	550		1563	8	8,4	1,25	27,36	1,76	28,00	1340			8,2
01.07.08	2880	13200	276	1855	8	8	1,2	28,60	1,85	30,00	1852			8,1
03.07.08	2885	13200	193	1313	8,1	9	1,5	26,70	1,73	27,50	1270		0,33	7,6
07.07.08	2880	13200	95	848	7,6	7,7	2,25	24,30	1,91	25,80	647			7,6
09.07.08	2880	13200	289	1848	6,9	4,9	2,50	25,40	3,00	28,30	479	18,31		5,7
11.07.08	2880	13200	163	667	7,8	7	3,50	26,00	3,00	28,00	300			8,0
15.08.08	414	14959	186	673	8,5	7,7	1,79	27,10	1,99	28,00	178			8,1

It was determined that COD input and output values were close to each other. The wastewater treatment plant outlet COD was reached up to very high levels, such as 300-1800 mg/L.

Table 3. Analysis values of wastewater treatment plant of petrochemical industry (August - September– October 2008/ Values in Application Time).

	Inlet Biological Treatment						Aeration TankA		Aeration TankB		Outlet Biological Treatment			
	Flow		COD		pH		DO	Temp	DO	Temp	COD	ahl Nitro	TotalP	pH
Date	Domestic m³/day	Outlet Flotation m³/day	Domestic m³/day	Outlet Flotation m³/day	Domestic m³/day	Outlet Flotation m³/day	mg/L	T°C	mg/L	T°C	mg/L	mg/L	mg/L	
20.08.08	477	13692	203	313	8,5	7,3	1,20	26,50	2,00	27,00	165	4,70	0,03	7,7
22.08.08	353	12897	225	1049	8,3	7,2	1,72	26,80	1,89	27,00	582	2,40		7,5
26.08.08	578	14135	256	184	8,3	7,2	2,45	27,00	1,88	27,00	176		0,07	7,3
27.08.08	568	12990	301	931	8,1	7,6	1,50	27,00	1,67	27,00	322	1,70		7,3
04.09.08	922	13706	244	705	8,3	7,4	1,10	25,00	2,27	25,90	394			7,9
05.09.08	845	13040	347	345	7,8	8,1	1,47	25,00	2,00	25,00	283	1,80	0,05	8,0
08.09.08	840	13616	347	1015	8,3	7,5	0,73	26,00	2,37	25,70	318	3,50		8,3
11.09.08	804	12332	296	1250	7,6	7,8	1,47	26,00	2,03	27,00	489			7,9
16.09.08	751	17781	344	396	8,5	8,5	1,65	25,00	1,79	26,10	302			8,4
17.09.08	937	13523	270	605	8,3	8,1	1,80	25,00	1,87	25,00	233			8,2
23.09.08	990	15842	331	568	7,7	7,3	1,25	23,00	1,10	23,30	391	8,20		7,6
25.09.08	949	16673	315	869	8	7	1,75	22,00	2,36	22,00	256		2,60	7,4
03.10.08	1296	17680	400	566	7,1	6,9	1,89	23,00	1,28	22,70	150			
08.10.08	918	14320	238	273	7,4	6,7	2,25	23,00	1,09	22,00	212			7,1
10.10.08	950	13466	219	335	7,2	8,2	2,11	22,00	1,49	22,00	150			6,7
14.10.08	660	18064	208	212	7	6,7	2,58	22,00	1,99	22,00	146			7,1
16.10.08	705	18458	192	262	7,2	7,1	2,41	22,00	1,44	22,00	74			6,2
20.10.08	582	16532	250	500	7,2	7,3	2,59	22,00	1,23	21,80	53	1,40		7,4
23.10.08	588	23740	238	352	7,1	5,3	2,27	22,00	2,41	22,00	154	1,20		7,4
27.10.08	667	18227	242	697	7,2	7,2	1,95	21,00	1,47	21,50	214			7,7

In September 2008, discharge COD concentrations were gradually stabilized and values decreased to 300-400 mg/L

Table 4. Petrochemicals industry – MLSS Analysis: August – September 2008.

Date	Aeration Tank A	Aeration Tank B
	MLSS (mg/L)	MLSS (mg/L)
04.08.08	424	401
06.08.08	766	729
14.08.08	985	705
15.08.08	1536	1182
18.08.08	759	622
20.08.08	**1087**	**659**
22.08.08	1426	1174
26.08.08	1356	1975
27.08.08	2061	2188
04.09.08	2050	2400
05.09.08	2320	3390
08.09.08	2230	1687
11.09.08	3540	2760
12.09.08	3570	2650
16.09.08	4910	4700
17.09.08	4470	4480
23.09.08	4240	4500
25.09.08	2860	3400
03.10.08	4320	3300
08.10.08	1630	2440
10.10.08	1810	1410
14.10.08	2110	1940
16.10.08	2220	2270
20.10.08	1620	1760
23.10.08	1220	1730
27.10.08	2750	1670
31.10.08	2970	1650

It was determined that mainly before the application, MLSS values seem to be under 1000 mg/L, but then in the second week MLSS values increased above 2000 mg/L, later it reached to 3000 – 4000 mg/L values.

The results of microbiological analysis are summarized in Table 5.

Table 5. Petrochemical industry - microbiological analysis.

Date	Protozoa Count					N	R	FB
	A	F	FSC	CC	SC			
22.08.2008	0	0	0	1	0	0	0	0
25.08.2008	0	0	0	11	0	0	0	1
26.08.2008	0	2	0	5	11	0	0	0
27.08.2008	0	2	0	0	21	0	0	0
28.08.2008	0	8	0	1	10	0	0	0
01.09.2008	0	0	0	0	7	0	0	0
02.09.2008	0	0	0	1	5	0	0	0
08.09.2008	0	0	0	2	3	0	0	0
09.09.2008	0	0	0	2	5	0	0	3
11.09.2008	0	1	0	2	13	0	0	0
12.09.2008	0	0	0	1	7	0	0	0
15.09.2008	0	4	0	7	9	0	0	0
16.09.2008	0	0	0	3	7	0	0	3
17.09.2008	0	7	0	4	2	0	0	7
18.09.2008	0	6	2	5	15	0	0	3
19.09.2008	0	2	0	5	6	0	0	0
22.09.2008	0	4	0	0	7	0	0	2
23.09.2008	0	0	0	1	5	0	0	2
24.09.2008	0	3	0	2	7	0	0	2
25.09.2008	0	4	0	0	7	0	0	2
26.09.2008	0	4	0	2	7	0	0	2
03.10.2008	0	3	0	1	3	0	0	2
06.10.2008	0	0	0	0	5	0	0	3
07.10.2008	0	0	0	0	4	0	0	2
08.10.2008	0	0	0	4	0	0	0	3
09.10.2008	0	1	0	0	14	0	3	4
10.10.2008	0	9	0	0	22	0	2	5
13.10.2008	0	3	0	2	7	0	3	3
16.10.2008	0	0	0	2	0	0	0	3
17.10.2008	0	0	0	0	0	0	1	7
20.10.2008	0	8	0	0	0	0	1	4
22.10.2008	0	0	0	0	0	0	0	7
23.10.2008	0	15	1	0	0	0	0	4
24.10.2008	0	11	1	0	2	1	1	4
27.10.2008	0	5	2	1	13	0	0	5
28.10.2008	0	4	1	18	0	0	0	9

A: Amoeba, F: Flagella, FSC: Free Swimming Ciliate, CC: Crawling Ciliate, SC:Stalked Ciliate, N: Nematode, R: Rotifer, FB: Filamentous Bacteria

According to protozoan analysis, it was observed that number of stalked and crawling ciliates increased rapidly on the following days of practice.

Free floating ciliate *Lionotus* spp. was seen very rarely in the flocs, usually seen more frequently in fluid. While *Paramecium* spp. were not seen in the flocs, they were found swimming closely to the flocs and giving the appearance like pulling the flocs. According to the observations on *Chilodonella unicinata*, it was generally found swimming in the fluid. Crawling ciliates were usually seen on the floc particles.

Epistylis spp. in the stalked ciliate group, were seen with their ovoid heads in the shape of bell. Colonies were identified as a four or seven headed stems. It was observed that *Vorticella* spp. had more rounded and broad structure than *Epistilis* spp. *Vorticella* spp. were not seen in the form of colony. A single stalk with one head was found attached on floc particles. In addition, the cilia in mouth part of the head were seen very clearly.

Amoeba was not seen during the application. Beginning from the second week an increase was determined in the flagella number. In spite of the decline in the number of stalked ciliates, they were still dominant in biomass.

A rotifer called *Philodina* spp. were observed with 400x magnification of the microbiological analysis, in some samples which were taken from petrochemical industry. Nematodes were observed with a 40x objective and seen that they were moving their bodies in the space between the particles back to forward. Segments have not been seen in their bodies. They were much thicker than the filamentous bacteria and the organelles in the bodies could be observed with 100x magnification.

Nematodes, rotifers and filamentous bacteria were observed very few during the test period.

Discussion

Selected adapted bacteria culture dosage was applied in different concentrations, according to wastewater flow rate and inlet COD values to the aeration basins, in the selected application area of petrochemical industry.

Dosing of adapted bacteria cultures was applied in two parts as the basis of shock and maintenance dosages. A certain period of time passes for bacteria to adapt and to achieve the maximum growth rate and biological activity. This period is much shorter for adapted bacteria cultures than the naturally occuring bacteria, though, further shortening of this period with shock dosage, it is possible to form a biomass rapidly and effectively and to achieve high treatment performance (Novozymes Biologicals, France). With the maintenance dosage, it is aimed to support the intensive biomass which has high treatment capacity, developed as the result of shock dosage and to protect the performance with continuous renewal. The dosing schedule can be changed depending on the application results (Novozymes Biologicals, France).

Before application, inlet COD concentrations to the biological treatment (outlet flotation unit - oily wastewater's COD value) and the discharge of COD values were very close to each other in petrochemical industry. The discharge of COD values began to fall with the beginning of the application, despite an increase was seen in inlet COD values later, outlet values of COD were saved low.

Before application of adapted bacteria cultures in biological wastewater treatment system (between 27.06 and 15.08.2008) inlet COD concentration showed fluctuations and COD removal rate was recorded to be low. It was recorded that COD inlet and outlet values were close to each other. The wastewater treatment plant outlet COD reached to very high levels, such as 300-1800 mg/L.

After the application started on 20.08.2008 with adapted bacteria cultures, outlet COD values began to change. In September 2008, during the application COD discharge, the concentration slowly stabilized and decreased to 300-400 mg/L. In the month of October 2008, COD discharge values were further improved and became well stabilized below the range of 200 mg/L, or even lower as 50 to 70 mg/L.

As shown in Table 3, effluent COD values became relatively independent from entry by late October and reached stable values. In this case, one of the main objectives of the adapted bacteria culture application, "to get stabilize discharge limits from independent influent parameters" was substantially achieved. Consequently the plant had very different processes and from time to time complex chemical substances entered to the biological treatment plant, so inlet COD values vary. Because of this reason, our first aim of this study was to develop a biomass which can balance this situation and withstand on toxic shocks as much as possible. However, also as seen in MLSS (Table 4) and microbiological analysis (Table 5), keeping biomass healthy with consistence in such a complex wastewater in this kind of facility can be unreliable. COD values reached by outlet of treatment plant and added adapted bacteria cultures showed that they could balance MLSS concentration in the aeration basins also in this type of complex wastewater.

The values of MLSS were recorded to be quite low in the pre-application process in August. In the beginning of September MLSS concentrations increased in both of A and B tanks, but also sudden fluctuations were observed. For example, decreases of MLSS values were recorded in late September and early October. Eventually, since all the conditions cannot always be controlled, it is not possible to prevent the unexpected MLSS falls. However, in spite of those changes in MLSS concentrations, the effluent COD did not increase, yet declined in some cases. This is a sign of a healthy biomass which could be developed.

After the application processes of adapted bacteria, the microbiological analysis chart (Table 5) was examined, and stalked ciliates were seen as they developed well and very quickly (especially in October). When stalked ciliates being dominant, it can be understood that active sludge can settle well, improvement in removing of organic matters and provided favorable conditions for nitrification.

As it is known, during the adjusting of poor operating conditions, an extreme increase can be seen in flagellate numbers (Jenkins *et al.* 2004). For this reason, the number of flagellates increased in October 2008 showed that biomass began to improve itself in the microbiological aspects. However, in the case of the number of bacteria increased, the amount of flagellates would decrease (Cutler and Crump 1920; Environmental Leverage Inc. 2008). In spite of the toxic conditions of wastewater, the efficiency of wastewater treatment was maintained. In this case, it can be explained that implemented adapted bacteria cultures are more resistant to toxic loads.

We were not able to obtain detailed information on the status of the biomass with performing chemical analysis alone. However, during the study of the microbiological analysis, despite a variety of adverse conditions caused by components of toxic wastewater, the bacterial cultures which were adapted to this type of wastewater showed us clearly the role of a healthy biomass formation during biological treatment.

Monitoring the quality of wastewater treatment with microbiological analysis in adapted bacteria culture applications is a way to follow the biological system. However, a study of this practice in Turkey has not been carried out. Adapted bacterial cultures in wastewater treatment is a matter in terms of "Recognizing that the every industrial institution as an individual field", wastewater pollution in the natural environment will be prevented by biological ways. Regarding the findings of this study, adapted bacterial cultures have shown the availability of specific purposes for providing effective treatment. Future studies on this subject may focus on better treatment with the use of adapted bacterial cultures investigated in more complex wastewaters of various industrial organizations, while microbiological analysis should be given priority. Continuation of these studies, indigenous cultures which can be developed in our country, take place in treatment process and output values will be available more quickly of the biological treatment that does not cause pollution into sea, river and lake that generate the discharge points.

Adapte bakteri kültürlerinin su kalitesi üzerinde iyileştirici etkileri: Petrokimya endüstrisi örneği

Özet

Bu çalışmada, toksik ve kalıcı organik kimyasal maddeler içeren endüstriyel atık suların biyolojik arıtım tesislerinde tam arıtımın yapılamadığı durumlarda, seçimli adapte bakteri kültürlerinin kullanımı ile arıtma tesisinin biyolojik havuzundaki biyokütlenin desteklenip güçlendirilmesiyle atık suların etkin biçimde arıtılması araştırılmıştır. Bu amaçla, çalışma alanı olarak petrokimya endüstrisi seçilmiştir. Biyolojik arıtma tesislerinin veriminin belirlenmesi amacıyla fiziksel, kimyasal analizler (debi, çözünmüş oksijen, sıcaklık, kimyasal oksijen ihtiyacı (KOİ), tam karışımlı askıda katı madde (MLSS), fenol, amonyum azotu, organik azot, fosfor, pH) yanında, ağırlıklı olarak mikrobiyolojik analizler (indikatör mikroorganizma türleri) yapılmıştır. Petrokimya endüstrisi biyolojik atık su arıtma tesisinde uygulama sırasında elde edilen mikrobiyolojik veriler ile, uygulama öncesinde ve sırasında kaydedilen kimyasal veriler adapte bakteri uygulamasının "giriş değerlerinden bağımsız stabil deşarj değerlerinin elde edilmesinin" gerçekleştiğini göstermiştir. Bu çalışmanın bulguları, adapte bakteri kültürlerinin biyolojik olarak atık suların arıtılması sırasında, kolaylıkla sağlıklı biyokütle oluşturmaları ve etkin arıtma sağlamaları konularında kullanılabilirliğini göstermiştir. Bu yöntemin sağlıklı biyokütle geliştirmek için bu tip atık sularda uygulanması tavsiye edilebilir.

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