İSTANBUL TECHNICAL UNIVERSITY ★ INSTITUTE OF SCIENCE AND TECHNOLOGY

SIMULATION AND PERFORMANCE OF BIOFILTER WITH ISOLATED AND CHARACTERIZED BENZYL ALCOHOL DEGRADING BACTERIA

M.Sc. Thesis by
Oktay KARAŞ, B.Sc.

Department : Environmental Engineering
Programme: Environmental Biotechnology

JANUARY 2007

İSTANBUL TECHNICAL UNIVERSITY ★ INSTITUTE OF SCIENCE AND TECHNOLOGY

SIMULATION AND PERFORMANCE OF BIOFILTER WITH ISOLATED AND CHARACTERIZED BENZYL ALCOHOL DEGRADING BACTERIA

M.Sc. Thesis by
Oktay KARAŞ, B.Sc.
(501031809)

Date of submission : 25 December 2006
Date of defence examination: 24 January 2007

Supervisor (Chairman): Assoc. Prof. Dr. Emine Ubay ÇOKGÖR
Members of the Examining Committee Prof.Dr. Z. Dilek HEPERKAN (ITÜ.)
Assoc. Prof.Dr. Kadir ALP (ITÜ.)

JANUARY 2007

PREFERENCES

I would like to express my special thanks and appreciation to Prof. Dr. rer. Nat. Karl Heinrich Engesser, Lab-supervisor: Dipl.-Ing. Niko Strunk, Assoc.Prof.Dr. Emine Ubay Çokgör and the other members of teaching staff of Program of WAREM (Water Resource Engineering and Management) and Department of Biological Air Purification at Institute of ISWA (Abteilung Biologische Abluftreinigung beim Institut für Siedlungswasserbau, Wassergute und Abfallwirtschaft) at Universität Stuttgart and the other members of teaching staff of Department of Environmental Biotechnology / ITU for their invaluable assistance and contribution to have this opportunity in improving my education.

I am grateful to my family who spent long and tedious days waiting for me to finalize of my study.

January, 2007 Oktay KARAŞ

CONTENTS

LIST OF ABBREVIATIONS

BAL	: Benzyl alcohol
BAD	: Benzyl aldehyde
NaB	: Sodium benzoate
Ca 1000	: Calcium Nitrate Solution, Concentrated as 1000 folds
HPLC	: High Pressure Liquid Chromatography
MM	: Minimal Medium
OD_{546}	: Optical Density at 546 nm
PP20	: Buffer Solution, Concentrated as 20 folds
SL100	: Mineral Salt Solution, Concentrated as 100 folds
NB	: Nutrient Broth Solution

LIST OF TABLES

LIST OF FIGURES

SIMULATION AND PERFORMANCE OF BIOFILTER WITH ISOLATED AND CHARACTERIZED BENZYL ALCOHOL DEGRADING BACTERIA

SUMMARY

In this study, simulation and performance of lab-scale biofilter for waste air (contaminated by Benzyl alcohol) were examined. Enrichment of Benzyl alcohol, Benzyl aldehyde and Sodium benzoate degrading bacteria, which were isolated from the soil, sediment of a creek and the soil around a tank station, were achieved. Pure strains could be obtained. Their growth kinetics was simultaneously performed by means of UV spectrophotometer and HPLC. Biochemical short tests (i.e. oxidase, catalase tests), gram (-) identification test (BIOLOG test) and identification of bacteria by API 20 NE test were performed. Sodium benzoate, Benzyl alcohol and Benzyl aldehyde calibration curves were obtained by HPLC analysis. The generation rate and metabolism rate were also determined. The species were used to prepare their pure liquid cultures. These liquid cultures were inoculated into lab-scale biofilter.

The main part of this study was simulation and observation of lab-scale biofilter for waste air (contaminated by Benzyl alcohol). For this purpose, the biofilter was set and run under various conditions.

ISOLE EDILEN VE TANIMLANAN BENZİL ALKOL GİDEREN BAKTERİLER İLE LAB ÖLÇEKLİ BİOFİLTRENİN PERFORMANS VE DAVRANIŞ KRİTERLERİNİN BELİRLENMESİ

ÖZET

Bu çalışmada benzyl alkol ile kirlenmiş kirli havanın temizlenmesinde kullanılan lab ölçekli biofiltrenin performans ve davranış karakterleri degerlendirilmiştir. Benzil alkol, benzyl aldehid ve sodyum benzoat gideren bakteriler elde edilmiş ve sayıları arttırılmıştır. Bu bakteriler toprak, dere çökeltileri ve benzin istasyonlarının bulundugu topraktan elde edilmiştir. Substrata özgü saf bakteriler elde edilmiştir. Bu bakterilerin büyüme ve gelişme kinetikleri UV spektrofotometresi ve HPLC cihazları ile izlenmiştir. Bakteriler üzerinde biyokimyasal kısa testler (Oksidaz ve Katalaz), gram (-) tanımlama (BIOLOG test) ve API 20 NE ile tanımlama testleri uygulanmıştır. Benzil alkol, benzyl aldehid ve sodyum benzoat kalibrasyon eğrileri HPLC analizi ile elde edilmiştir. Populasyonlarını ne kadar sürede iki katına çıkardıkları ve substrat metabolizma zaman tesleri yapılmıştır. Elde edilen bakteriler saf sıvı kültür hazırlanırken kullanılmıştır. Bu hazırlanan saf sıvı kültürler daha sonra lab ölçekli biyofiltrede aşılayıcı olarak kullanılmıştır.

Bu çalışmanın asıl amacı benzyl alkol ile kirlenmiş kirli havanın temizlenmesinde kullanılan lab ölçekli biofiltrenin performans ve davranış karakterleri degerlendirilmesidir. Bu amaç için biyofiltre oluşturulmuş ve farklı koşullarda çalıştırılmıştır.

1. INTRODUCTION

1.1. State-of-The Art of Science and Technology

When world is industrializing, it faces with more severe environmental problems. Our air, water and soil resources are drastically affected by this pollution. Industry and human beings discharge every day huge amount of wastewater. Moreover, they emit uncontrolled waste gases into our atmosphere.

In wastewater treatment and waste air purification, microorganisms are more often used as a solution those days due to its cost and its relevancy to environment. Pollutants are transformed to comparably less harmless products during the purification process by their metabolisms. Most of these microorganisms are naturally occurring. There are also some DNA modified microorganisms. But they are generally less powerful than natural ones. After micro organisms adapt to the applied environment, they degrade the pollutants. In air purification process compared to that of water, less biomass occur generally. The difference in the mass is converted generally into heat energy. The strains existing in the treatment process should be examined. By this way, unique microbial communities for pollutant treatment are optimised and developed.

The main part of this study was simulation of lab-scale biofilter for waste air (contaminated by Benzyl alcohol). For this purpose, the biofilter was set and run under variable conditions. In order to achieve this aim, all needed microbiological laboratory methods and skills as described in the abstract were used.

2. LITERATURE SURVEY

2.1 Substrate Characteristics, Usage And Legislation

The formula of Benzyl alcohol is $C_6H_5CH_2OH$. It is an aromatic organic compound. It is a colourless liquid. It is natural constituent of a variety of essential oils. Its polarity, low toxicity and low vapour pressure are its most important characteristics. It is partially soluble in water (i.e. 4 g / 100 mL). It is completely miscible in alcohols and ether. It is used in inks, paints, lacquers, epoxy resin coating, soap, perfume, flavour, pharmaceuticals and photography industries. It has bacteriostatic and antipruritic properties. Its world production was around at 6600000 tons in 2000 (www.uk.chemdat.info/mda/uk)

The formula of Benzyl aldehyde is C_6H_5CHO. It is the simplest aromatic aldehyde. It is a colourless liquid. It smells like almond. Due to this effect, it is used as food flavouring substance. It is used in the synthesis of other organic compounds, ranging from plastic additives to pharmaceuticals. It is also used in perfume and dyes industries. The usage of Benzyl aldehyde as a dyestuff in 1992 was estimated at amount of 467 000 tons (www.uk.chemdat.info/mda/uk)

The formula of Benzoic acid is C_6H_5COOH. It is the simplest aromatic carboxylic acid. It is colourless crystalline solid. It is used mostly as a food preservative. It is also used for synthesis of many other organic substances. The production amount was estimated as 126000 tons / year in United States (www.uk.chemdat.info/mda/uk).
Regarding to emission limits, organic substance expressed as TOC should not be exceeding daily average value of 20 mg/m^3. Total dust should not be exceeding daily average value of 10 mg/m^3. Organic substance expressed as TOC should not be exceeding half hourly average value of 40 mg/m^3. Total dust should not be exceeding daily average value of 30 mg/m^3. Those values are from European Legislations (http://europa.eu/scadplus/leg/en/lvb/l28029b.htm).

Regarding to hazard codes, risk statements and safety statements data are achievable in part of the list of substance characteristics (Table 2.1).

Table 2.1. Substrate Characteristics

	Benzyl alcohol	**Benzaldehyde**	**NaBenzoate**
Synonym	Benzenemethanol	Artificial essential oil of almond	Benzoic acid sodium salt
Molecular Formula	C6H5CH2O	HC_6H_5CHO	C_6H_5COONa
Molecular Weight	108.14	106.12	144.10
CAS Number	100-51-6	100-52-7	532-32-1
Properties			
vapor density	3.7 (vs air)	3.7 (vs air)	
vapor pressure	13.3 mm Hg (100 °C) 3.75 mm Hg (77 °C)	4 mm Hg (45 °C)	pH 7.0-8.5 (25 °C, 1 M in H_2O)
autoignition temp.	817 °F	374 °F	
refractive index	n20/D 1.539 (lit.)	n20/D 1.545(lit.)	
Bp	203-205 °C (lit.)	178-179 °C(lit.)	
Mp	-16-13 °C (lit.)	−26 °C(lit.)	>300 °C(lit.)
Fp	201 °F		
Density	1.045 g/mL at 25 °C (lit.)	1.045 g/mL at 25 °C(lit.)	
Solubility	1g/25 ml	0,6 wt@20°C,1,5 wt@20°C	H_2O: 1 M at 20 °C,
Safety			
Hazard Codes	Xn	Xn	
Risk Statements	20/22	22	
Safety Statements	26	24	
WGK Germany	1	1	1
RTECS	DN3150000	CU4375000	

2.2 Survey On Toluene And Benzyl alcohol Degrading Bacteria

Xylene monooxygenase of *Pseudomonas putida* mt-2 catalyzes the methylgroup hydroxylation of toluene and xylenes. Xylene monooxygenase was found to catalyze the oxygenation of toluene, pseudocumene, the corresponding alcohols, and the corresponding aldehydes. For all three transformations ^{18}O incorporation provided stong evidence for a monooxygenation type of reaction, with *gem*-diols as the most likely reaction intermediates during the oxygenation of benzyl alcohols to benzaldehydes. To investigate the role of benzyl alcohol dehydrogenase (XylB) in the formation of benzaldehydes, *xylB* was cloned behind and expressed in concert with *xylMA*. In comparison to *E. coli* expressing only *xylMA*, the presence of *xylB* lowered product formation rates and resulted in back formation of benzyl alcohol from benzaldehyde. In *P. putida* mt-2 XylB may prevent the formation of high concentrations of the particularly reactive benzaldehydes. In the case of high fluxes through the degradation pathways and low aldehyde concentrations, XylB may contribute to benzaldehyde formation via the energetically favorable dehydrogenation of benzyl alcohols. XylMA as an enzyme can catalyze the multistep oxygenation of toluenes (Bühler et al., 2000).

Benzyl alcohol dehydrogenase and benzaldehyde dehydrogenase, two upper pathway enzymes, exhibit very broad substrate specificities and transform unsubstituted substrates and m- and p-methyl-, m- and p-ethyl-, and m- and p-chloro-substituted benzyl alcohols and benzaldehydes, respectively, at a high rate.

Chemicals containing one or more benzene rings can be mineralized by soil and sediment bacteria. In many instances the genetic information for the biodegradative process is harbored by plasmids which act as efficient vehicles for the spread of such information. The TOL plasmid of Pseudomonas putida is the most extensively characterized catabolic plasmid; it encodes enzymes for the mineralization of toluene, m- and p-xylene, m-ethyltoluene, and 1,3,4-trimethylbenzene. In the degradation of these compounds, the methyl group at carbon 1 in the aromatic ring is sequentially oxidized to yield the corresponding carboxylic acid (upper pathway). The carboxylic acid is then oxidized to its corresponding catechol, which undergoes meta fission to produce a semialdehyde which is further transformed into products (pyruvate plus aldehydes)

that are finally transformed by chromosomally encoded enzymes into Krebs cycle intermediates (Abril et al., 1989).

Agrocybe aegerita, a bark mulch- and wood-colonizing basidiomycete, was found to produce a peroxidase that oxidizes aryl alcohols, such as veratryl and benzyl alcohols, into the corresponding aldehydes and then into benzoic acids. The enzyme also catalyzed the oxidation of typical peroxidase substrates, such as 2,6-dimethoxyphenol or 2,2_-azinobis-(3-ethylbenzothiazoline-6-sulfonate). *A. aegerita* peroxidase production depended on the concentration of organic nitrogen in the medium. The optimum pH for the oxidation of aryl alcohols was found to be around 7, and the enzyme required relatively high concentrations of H_2O_2 (2 mM) for optimum activity (Ullrich et al., 2004).

Most aerobic biodegradation pathways for hydrocarbons involve iron-containing oxygenases. In iron-limited environments, such as the rhizosphere, this may influence the rate of degradation of hydrocarbon pollutants. We investigated the effects of iron limitation on the degradation of toluene by *Pseudomonas putida* mt2 and the transconjugant rhizosphere bacterium *P. putida* WCS358(pWWO), both of which contain the pWWO (TOL) plasmid that harbors the genes for toluene degradation. The results of continuous-culture experiments showed that the activity of the upper-pathway toluene monooxygenase decreased but that the activity of benzyl alcohol dehydrogenase was not affected under iron-limited conditions. In contrast, the activities of three *meta*-pathway (lower-pathway) enzymes were all found to be reduced when iron concentrations were decreased. Additional experiments in which citrate was used as a growth substrate and the pathways were induced with the gratuitous inducer *o*-xylene showed that expression of the TOL genes increased the iron requirement in both strains. Growth yields were reduced and substrate affinities decreased under iron-limited conditions, suggesting that iron availability can be an important parameter in the oxidative breakdown of hydrocarbons (Dinkla et al., 2001).

It is analyzed metabolic interactions and the importance of specific structural relationships in a benzyl alcohol-degrading microbial consortium comprising two species, *Pseudomonas putida* strain R1 and *Acinetobacter* strain C6, both of which are able to utilize benzyl alcohol as their sole carbon and energy source. The organisms were grown either as surface-attached organisms (biofilms) in flow chambers or as suspended cultures in chemostats. The numbers of CFU of *P. putida* R1 and *Acinetobacter* strain C6 were determined in chemostats and from the effluents of the

flow chambers. When the two species were grown together in chemostats with limiting concentrations of benzyl alcohol, *Acinetobacter* strain C6 outnumbered *P. putida* R1 (500:1), whereas under similar growth conditions in biofilms, *P. putida* R1 was present in higher numbers than *Acinetobacter* strain C6 (5:1). In order to explain this difference, investigations of microbial activities and structural relationships were carried out in the biofilms. Insertion into *P. putida* R1 of a fusion between the growth rate-regulated rRNA promoter *rrnB*P1 and a *gfp* gene encoding an unstable variant of the green fluorescent protein made it possible to monitor the physiological activity of *P. putida* R1 cells at different positions in the biofilms. Combining this with fluorescent in situ hybridization and scanning confocal laser microscopy showed that the two organisms compete or display commensal interactions depending on their relative physical positioning in the biofilm. In the initial phase of biofilm development, the growth activity of *P. putida* R1 was shown to be higher near microcolonies of *Acinetobacter* strain C6. High-pressure liquid chromatography analysis showed that in the effluent of the *Acinetobacter* strain C6 monoculture biofilm the metabolic intermediate benzoate accumulated, whereas in the biculture biofilms this was not the case, suggesting that in these biofilms the excess benzoate produced by *Acinetobacter* strain C6 leaks into the surrounding environment, from where it is metabolized by *P. putida* R1. After a few days, *Acinetobacter* strain C6 colonies were overgrown by *P. putida* R1 cells and new structures developed, in which microcolonies of *Acinetobacter* strain C6 cells were established in the upper layer of the biofilm. In this way the two organisms developed structural relationships allowing *Acinetobacter* strain C6 to be close to the bulk liquid with high concentrations of benzyl alcohol and allowing *P. putida* R1 to benefit from the benzoate leaking from *Acinetobacter* strain C6. We conclude that in chemostats, where the organisms cannot establish in fixed positions, the two strains will compete for the primary carbon source, benzyl alcohol, which apparently gives *Acinetobacter* strain C6 a growth advantage, probably because it converts benzyl alcohol to benzoate with a higher yield per time unit than *P. putida* R1. In biofilms, however, the organisms establish structured, surface-attached consortia, in which heterogeneous ecological niches develop, and under these conditions competition for the primary carbon source is not the only determinant of biomass and population structure (Christensen et al., 2002).

Toluene and the three isomers of xylene were completely mineralized to CO2 and biomass by aquifer-derived microorganisms under strictly anaerobic conditions. The

source of the inoculum was gasoline-contaminated sediment from Seal Beach, Calif. Evidence confirming that sulfate was the terminal electron acceptor is presented. Benzene and ethylbenzene were not degraded under the experimental conditions used. Successive transfers of the mixed cultures that were enriched from aquifer sediments retained the ability to degrade toluene and xylenes. Greater than 90% of 14C-labeled toluene or "4C-labeled o-xylene was mineralized to '4Co2. The doubling time for the culture grown on toluene or m-xylene was about 20 days, and the cell yield was about 0.1 to 0.14 g of cells (dry weight) per g of substrate. The accumulation of sulfide in the cultures as a result of sulfate reduction appeared to inhibit degradation of aromatic hydrocarbons (Edwards et al., 1991).

2.3 Biofiltration Of Toluene With The Fungus

Studies of biofiltration show that bacterial biofilms can degrade low concentrations of volatile organic compounds from off-gas streams. Recent work has demonstrated that biofilters containing fungi effectively eliminate volatile organic compounds, even under adverse environmental conditions such as low moisture content, low pH, and transient loadings. Previous results have shown that a biofilter inoculated with a fungal strain (reported as *Scedosporium apiospermum* TB1 but recently reclassified as *Paecilomyces variotii* CBS115145) reached and maintained high elimination capacities (EC) of toluene (higher than 200 g/m3 of biofilter/h, with a removal efficiency of 98%). The maximum EC of toluene, around 245 g/m3 of biofilter/h, was obtained with the same strain growing on porous ceramic rings. The biofilter exhibited bacterial contamination, but fungal activity was responsible for about 70% of total removal. Woertz et al. reported the performance of a biofilter containing *Exophiala lecanii-cornii* with an average toluene EC of around 80 g/m3 of biofilter/h and removal efficiencies greater than 95%. ECs up to 270 g/m3 of biofilter/h were attained for short periods. *Exophiala oligosperma* and *Paecilomyces variotii* have been recently reported to remove toluene with ECs of 55 and 80 g/m3 of biofilm/h. Five metabolic pathways for toluene are known in bacteria. Toluene is initially hydroxylated on either the methyl group or the aromatic ring by an oxygenase. In fungi, initial hydroxylation on both molecular sites has also been reported. Some zygomycetes and deuteromycetes were shown to hydroxylate toluene at the aromatic ring, and the intermediates *o*-cresol and *p*-cresol were identified. Weber et al. reported *Cladosporium sphaerospermum* growth

on toluene with initial hydroxylation on the methyl group. Recently, Prenafeta-Boldu´ et al. used isomeric fluorotoluenes as model substrates for toluene catabolism by five fungi that grow on toluene and two others that cometabolize toluene with glucose (*Cunningamella echinulata* and *Aspergillus niger*). With the toluene-utilizing fungi and *A. niger*, initial hydroxylation occurred only at the methyl group and resulted in fluorinated benzoates. In *C. echinulata*, hydroxylation was also initiated at the aromatic ring, and *o*-cresol and benzoate were detected. For toluene, it has been proposed that fungi preferentially hydroxylate the methyl group. Parallel pathways for the degradation of aromatic compounds have been shown elsewhere in fungi. Jones et al. reported that in the *p*-cresol oxidation by *Aspergillus fumigatus*, both the methyl group and the ring hydroxylation were involved. Also with *A. fumigatus*, Jones et al. showed that phenol degradation was initiated by both *o* and *p* hydroxylation. Similar results were found with *Paecilomyces lilacinus* on biphenyl degradation. Enzymatic activities have seldom been used to understand the macroscopic behavior of biofilters. However, several au- thors have correlated performance and metabolic measurements in bacterial biofilters. The main objective of this work was to evaluate the relationship between the enzymatic activity and the performance of a biofilter for toluene degradation inoculated with *Paecilomyces variotii* CBS115145. The toluene metabolic pathway was first elucidated and the main enzyme activities and degradation products were evaluated in mycelia obtained from liquid cultures and biofilters. In a long-term biofiltration experiment, enzymatic activities were measured on samples collected along the biofilter and compared with the experimental macroscopic toluene elimination capacity (Garcia Pena et al., 2005).

2.4 Biotrickling Filtration Principle

Biological treatment of contaminated air is an alternative technology for air pollution control nowadays. The principle is that a contaminated air flow is passed through a porous packed bed on which pollutant-degrading cultures are placed. Air biotreatment relies on microbial achievement for the degradation of waste compounds. Bioreactors for air pollution control is used in the treatment of dilute, high flow waste gas flows containing odors or volatile organic compounds (VOCs). Under desired conditions, the volatile or gaseous pollutants can be degraded to carbon dioxide, water and biomass. In the case of contaminants such as H_2S or reduced sulfur compounds, or

biodegradable chlorinated compounds, sulfate or chloride are additional by-products. Bioreactors for air pollution control treat many contaminants in a wide range of applications. The technology offers several advantages over traditional technologies such as incineration or adsorption. These include lower treatment costs, reduced environmental impact, and absence of formation of by-products such as nitrogen oxides (NOx) or spent activated carbon, low energy demand, no need for fossil fuel burning, and low temperature treatment. The two types of bioreactors for air pollution control are biofilters and biotrickling filters.

Biofilters work by passing a humid stream of contaminated air through a damp packing material, usually compost mixed with wood chips or any other bulking agent, on which pollutant degrading bacteria are naturally placed. Biofilters are simple and cost effective. They require low maintenance and are particularly effective for the treatment of odor and volatile compounds that are easy to biodegrade and for compounds that do not generate acidic by-products. Biofilters are increasingly used in industrial applications.

Biotrickling filters work in a similar way to biofilters, except that an aqueous phase is trickled over the packed bed, and that the packing is usually made of some synthetic or inert material, like plastic rings,open pore foam, lava rock, etc. The trickling solution contains essential inorganic nutrients such as nitrogen, phosphorous, potassium, etc. and is usually recycled. Biotrickling filters are more complex than biofilters but are usually more effective, especially for the treatment of compounds that generate acidic by-products, such as H_2S. They can be built taller than biofilters. Biotrickling filters are more recent than biofilters, and have not yet been fully used in industrial applications (Deshusses et al., 2002).

Biotrickling filters are biological scrubbers. The mechanisms is that contaminated air is contacted with an immobilized culture of pollutant degrading organisms in a packed bed. The processes reveals that elimination of the pollutant is the result of a combination of physico-chemical and biological phenomena. Understanding these phenomena is a key to the successful deployment of the technology.

In biotrickling filters, contaminated air is forced through a packed bed, either downflow or upflow. The packed bed is generally made of an inert material such as a random dump or a structured plastic packing, or less often, an open pore synthetic foam or lava

rocks. The packing provides the necessary surface for biofilm attachment and for gas-liquid contact. During treatment, an aqueous phase is recycled over the packing. It provides moisture, mineral nutrients to the process culture and a means to control the pH or other operating parameters. The system is continuously supplied with essential mineralnutrients such as nitrogen, phosphorus, potassium, and trace elements via a liquid feed. In general, most of the pollutant is biodegraded in the biofilm, but part may also be removed by suspended microorganisms in the recycle liquid. Possible biodegradation metabolites will leave the system via the liquid purge along with small amounts of biomass. Usually, less than 10% of the carbon-pollutant entering the system leaves via the purge.

Biotrickling filters work because of the action of the pollutant degrading microorganisms. In the case of the removal of hydrocarbon vapors, the primary degraders are aerobic heterotrophic organisms that use the pollutant as a source of carbon and energy. For H2S or ammonia removal, the primary degraders are autotrophes, and will use the pollutant as a source of energy, and carbon dioxide as source of carbon for growth. The removal of compounds such as dimethyl sulfide or dimethyl disulfide will require both autotrophes and heterotrophes to be present. In any case, the biotrickling filter will host a wide variety of microorganisms, similar to those encountered in waste water treatment operations. The microorganisms responsible for pollutant removal in biotrickling filters are usually aerobic because biotrickling filters are well aerated systems. However, it has been proposed that the deeper parts of the biofilm, where anaerobic conditions probably prevail, can be utilized to perform anaerobic biodegradation (e.g., reductive dechlorination, or NOx reduction) for the treatment of pollutants that are otherwise recalcitrant under aerobic conditions. Anaerobic treatment in aerobic biotrickling filters is still an experimental area.

A major fraction of the biofilm becomes inactive (mostly because of mass transfer limitations) as the biofilm grows, and active primary degraders only constitute a minor fraction of the total population in the biofilm. Secondary degraders feeding on either metabolites, biopolymers, or predators feeding on the primary degraders include bacteria, fungi, and higher organisms such as protozoa, rotifers, even mosquito or fly larvae, worms or small snails. The importance of higher organisms for the overall process should not be underestimated. They have been shown to play an important role in reducing the rate of biomass accumulation and in recycling essential inorganic

nutrients. As a matter of fact, comparison of traditional mineral growth media with biotrickling filter recycle liquid composition reveals that mostbiotrickling filters are operated under various degrees of inorganic nutrient limitation. The relationship between nutrient supply and biomass growth is discussed further in this chapter (Deshusses et al., 2002).

2.5 Biotrickling Filter Performance

2.5.1 Definitions and factors affecting performance

Operation and performance of biological reactors for air pollution control is generally reported in terms of removal efficiency, or pollutant elimination capacity as a function of the pollutant loading, or the gas empty bed retention time (EBRT). These terms are defined in Equations 2.1-2.4

$$\text{Removal}=RE=((C_{in}-C_{out})/C_{in})\times 100\ (\%) \quad \textbf{(2.1)}$$

$$\text{Pollutant Elimination Capacity}=EC=((C_{in}-C_{out})/V)\times Q\ (gm^{-3}h^{-1}) \quad \textbf{(2.2)}$$

$$\text{Empty Bed Retention Time}=EBRT=V/Q\ (s \text{ or } min) \quad \textbf{(2.3)}$$

$$\text{Pollutant Loading}=L=(C_{in}/V)\times Q\ (gm^{-3}h^{-1}) \quad \textbf{(2.4)}$$

where C_{in} and C_{out} are the inlet and outlet pollutant concentrations (usually in gm^3), respectively, V is the volume of the packed bed (m^3) and Q is the air flow rate ($m^3\ h^{-1}$). Pollutant concentrations are usually reported as mass per volume; conversion of volumetric to mass concentrations is done using the ideal gas law which reduces to Equation 2.5 at room temperature.

$$\text{Concentration}\ (gm^{-3})=\text{Concentration}(ppm_v)\times \text{molecular weight of pollutant}\ (gmol^{-1})/24{,}776 \quad \textbf{(2.5)}$$

It should be stressed that the elimination capacity and the loading are calculated using the volume of the packed bed and not to the total volume of the reactor. Depending on the reactor design, the volume of the packed bed volume will be about 40-90% of the total reactor volume. Also, the EBRT is calculated on the basis of the total volume of packed bed (Equation 2.3). The actual gas residence time will be lower depending on the porosity of the packing, the dynamic liquid hold-up and the amount of biomass attached to the packing. The porosity of packing ranges from about 50% (lava rock) to

95% (all random or structured packings), the liquid holdup is usually less than 5% of the bed volume, and biomass may occupy 5% to 30% of the bed volume. Hence, the actual gas residence can be less than half the EBRT.

It is usual to report the performance as a function of the load, i.e., inlet concentration *air flow, rather than the concentration. This enables comparison of systems of different sizes operated under different conditions. One underlying assumption is that the performance depends only on the pollutant load, hence, that low concentrations high flow rates conditions lead to similar elimination capacities as high concentrations-low flow rates. This assumption is generally valid because the pollutant concentrations commonly encountered in biotrickling filters are high enough for the micro-kinetics to be of zero order. This is no longer true at very low pollutant concentrations (typically below 0.05 - 0.1 g m^{-3}), in particular for pollutants with high Henry's law coefficients, because first order kinetics will prevail in the biofilm resulting in a reduction of the maximum elimination capacity.

There are essentially three operating regimes.

1. Low loading, also called first order regime.The elimination capacity and the loading are identical and the pollutant is completely removed. The biotrickling filter is operated well below its maximum elimination capacity. The performance increases proportionally with the loading.

2. Intermediate range. Breakthrough of the pollutant occurs. With higher inlet concentration or higher air flow rates, the elimination capacity increases, but to a lesser extent than the loading.

3. High loading, also called zero order regime.The biotrickling filter is operated at its maximum elimination capacity. Increases in pollutant concentration or of the air flow rate do not result in further increases in elimination capacity, the removal efficiency decreases. For the evaluation of biotrickling filter performance, one should consider both the maximum elimination capacity and the removal efficiency. For practical reasons, academic research is mainly concerned with the maximum elimination capacity or with high performance, which occur at relatively high pollutant concentration and often less than ~90% removal efficiency. On the other hand, reactor design for industrial application often needs to meet a certain discharge requirement, or achieve a high removal percentage. Thus there might be some challenges in extrapolating

research data for reactor design. In this context, the critical load defined as the maximum loading before the removal deviates significantly from the 100% removal line is a valuable parameter. But there are limitations to the use of the critical loading. It is relatively sensitive to the pollutant inlet concentration, thus extrapolation of low flow-high concentrations to high flow low concentration should be avoided (Deshusses et al., 2002).

2.5.2 Examples of biotrickling filter performance

Research over the past ten years has greatly broadened the range of pollutants that can be treated in biotrickling filters, including volatile organic compounds (VOCs), chlorinated hydrocarbons, reduced sulfur compounds, and compounds containing nitrogen. Maximum elimination capacities generally are in the range of 5-200 g m^{-3} h^{-1}. Although many factors influence performance, a few general comments can be made. As biotrickling filters rely on microorganisms as the catalysts for pollutant conversion, biodegradability of the pollutant is of prime importance. Decreasing biodegradability causes lower elimination capacities and/or longer periods of adaptation. The use of specially acclimated or enriched microorganisms may be considered in these cases. Equally important is the accessibility of the pollutant to the microorganisms. The overall rate of pollutant removal may be limited by mass transfer rate of the pollutant into the biofilm, which depends mainly on the pollutant's air-water partition which is in turn best described by the Henry coefficient. Mass transfer limitation leads to a biofilm not completely saturated with the pollutant, hence pollutant concentrations in the biofilm are below those required for maximum biological activity. Means to improve the overall mass transfer rate in biotrickling filters include the selection of packing materials with a high specific surface area and intermittent trickling to reduce the thickness of the water film on the biofilm.

Many different types of packing materials have been used in biotrickling filters, and research in this area is still ongoing. The packing should combine a high porosity to minimize the pressure drop across the reactor and a high specific surface area to maximize biofilm attachment and pollutant mass transfer. Other factors to consider for a packing include water holding capacity, structural strength, surface properties, weight, stability over time, and cost. Reaction conditions in the biotrickling filter can be optimized by controlling the pH, the concentrations of nutrients and metabolic end-

products in the recycle liquid. Many biotrickling filters are equipped with a pH control, and with automatic water/nutrient addition to control ionic strength. The optimum pH depends on the process culture. Most VOC-removing biotrickling filters are operated at a near neutral pH. On the other hand, H_2S oxidizing microorganisms such as Thiobacillus sp. are acidophilic and show maximum activity at low pH. pH values as low as 1-2 are not uncommon in biotrickling filters treating H_2S vapors. Treatment of sulfur and chlorinated compounds will result in the accumulation of sulfate and chloride in the recycle liquid, respectively. These salts will inhibit biodegradation if certain concentrations are exceeded, and frequent supply of fresh water and purge of the recycle liquid is required to prevent accumulation of inhibitory concentrations. The dilution rate can be controlled by continuous measurement of the conductivity of the recycle liquid or by using ion selective electrodes (Deshusses et al., 2002).

2.6 Biomass Growth In Biotrickling Filters

2.6.1 Growth kinetics

Clogging of biotrickling filters by growing biomass is one factor that has markedly slowed down the implementation of biotrickling filters at the industrial scale. A better understanding of biomass growth in biotrickling filters is warranted. In general, pollutants are used by the primary degraders to produce new biomass and to generate energy for maintenance. These processes have been extensively investigated in batch or continuous monocultures. The situation is much more complicated in biotrickling filters where a complex ecosystem exist. In a first approximation, neglecting heterogeneities and mass transfer effects, one can write that the rate of pollutant degradation depends on the intrinsic growth rate of the active fraction of the primary degraders (X_1) and their maintenance requirement, as in Equation 2.6.

$$EC=[(\mu/Y_{X/S})+m] \times X_{1(\text{active fraction})} \qquad \textbf{(2.6)}$$

Where μ is the specific growth rate of the primary degraders, $Y_{X/S}$ is the biomass yield, m the maintenance energy requirement, and X_1(active fraction) is the biomass content of active primary degraders per volume of reactor.

The specific growth rate of the active fraction of the primary degraders can be expressed using a modified Monod type equation,

$$\mu=[(\mu_{max}xS)/(K_s+S)]x[N/(K_{SN}+N)]x[O(K_{SO}+O)]x[I/(1+(I/K_I))] \quad \textbf{(2.7)}$$

Where S is the pollutant and substrate, N is any nutrient, O is the oxygen, and I any inhibitor, and Ks, Ks_N, Ks_O, and K_I are the respective half-saturation and inhibition constants.

A similar equation can be written for all the species (or group of species) present in the system. Each will have one or several specific substrates, specific kinetic constants, and thus a specific growth rate. The overall rate of biomass accumulation is the sum for all the different species (designated by the indices i) of the growth rate minus death and lysis (d term), the predation by other species and the wash-out via the recycle liquid purge. This is expressed in Eq. 2.8.

$$\text{Rate of biomass accumulation}=\Sigma((\mu_i-d)\ x\ X_i-\text{Predation}_i-\text{Wash out}_i) \quad \textbf{(2.8)}$$

Equations 6-8 are highly simplified since they do not take local heterogeneities into account. Still they define a number of parameters that are impossible to determine. A possible solution is to split the process culture into large classes of organisms, such as primary degraders, secondary degraders, predators, etc. and use lumped kinetic parameters. This is an area of current research. Even so, Eq.s 2.6-2.8 reflect the fact that the pollutant elimination and the observed biomass growth are interrelated in a complex manner. The equations further allow development of biomass control strategies for biotrickling filters. This is discussed in the next section (Deshusses et al., 2002).

2.6.2 Strategies for controlling biomass growth

Examination of Equations 2.6-2.8 suggests several possible approaches to controlling biomass growth. Attempts can be made to reduce the overall rate of biomass accumulation (Equation 2.8) by either reducing the specific growth rate or increasing death and lysis. Several means have been investigated. Other options include increasing predation, washingout or otherwise periodically removing the excess biomass. These are briefly discussed.

The first option to prevent clogging is the reduction of the biomass accumulation rate or of the specific growth rate (Equations 2.7). The challenge is to reduce biomass accumulation, while maintaining a high pollutant removal rate (Equation 2.6), since growth and pollutant elimination are often tightly linked. This can be achieved by reducing the biomass yield coefficient ($Y_{X/S}$) and/or increasing the maintenance requirements (m). Growth, biomass yield, death and lysis, activity and maintenance are interrelated parameters reflecting general cell metabolism and as such they are difficult to influence independently. Various attempts to reduce the specific growth rate in biotrickling filters. include limiting the supply of nutrients essential for growth (N or K), the use of nitrate as a nitrogen source instead of ammonium, the addition of compounds such as NaCl in concentrations that partially inhibit microbial growth, etc. In general, these strategies also result in reduction of microbial activity, thus they lower reactor performance. Hence, larger reactors will be required to treat the same volume of waste gas, which will increase the capital costs. An interesting option is the use of organisms with lower biomass growth rates and yields such as fungi. Interestingly, under similar conditions, fungi have shown a higher removal rate and a lower biomass accumulation rate than bacteria in toluene degrading biotrickling filters operated under nutrient limiting conditions. The second option is to stimulate predation of the process culture by higher organisms such as protozoa, possibly even larger organisms such as larvae, small snails or other biomass-eating organisms. This is a promising approach since it will not lead to a reduction of the performance, and will not result in excess biomass to be disposed off, as for the methods discussed in the next paragraph. The challenge is that higher organisms may be difficult to control and/or to maintain in the biotrickling filter. This is an area of development, and advances are expected in the near future. The last option to prevent clogging is to remove the excess biomass. This is usually done periodically rather than continuously, because shear by the trickling liquid during normal operation is not sufficient to remove substantial amounts of attached biomass. Hence, the recycle liquid only contains a low concentration of biomass and increasing blow down does not wash-out much biomass. When periodical removal of biomass is chosen, the biotrickling filter is best operated at a high elimination capacity, and biomass is allowed to accumulate up to a given point where remedial action is required. From a cost perspective, the capital costs will be lower because a smaller reactor will suffice, but clogging will necessitate frequent cleaning, thus increasing the operating costs. Removal of biomass can be done either physically or

chemically. Physical removal of biomass relies on biofilm detachment by high shear forces. This can be done by backwashing the reactor, or by periodical stirring of the packed bed. Although these techniques result in prolonged, stable biotrickling filter operation, certain drawbacks exist. Chemical removal of biomass is a simpler operation as no major changes of the reactor configuration are required. In this procedure, a chemical solution is recycled over the packing using the existing system for liquid recycling. A stable toluene-degrading biotrickling filter was obtained by periodic washing of the packing with a NaOH solution for 3 hours. A post-treatment with HCl was needed to restore the pH to a neutral value. Other chemicals such as sodium hypochlorite and hydrogen peroxide may be more effective in removing biomass, but they are also more toxic to the microbial population. This could potentially slow down the restart of the reactor.

Unfortunately, all biomass control strategies have only been investigated in the laboratory and no experience is available from industrial-scale biotrickling filters. This is because most full-scale biotrickling filters have been designed for applications with low potential for clogging. In the future, design and operation of biotrickling filters will need to find the optimum between operation of large, low-performance biotrickling filters that do not require biomass removal, and small, high performance biotrickling filters with high potential for biomass accumulation (Deshusses et al., 2002).

3. MATERIALS AND METHODS

3.1. Optical Density (OD_{546}) Measurements by Photometer

The absorption of solution of microorganisms is measured at 546 nm by photometer. It is called as optical density of microorganism. Before the measurement, spectrophotometer should be calibrated with relevant solution.

In this method, Spectrophotometer, Polystyrene disposable or Quartz cuvettes, 1,5 ml, 12,5*12,5*45 mm, assorted by one cavity number, Eppendorf pipette and Cultures are used as materials.

The spectrophotometer is calibrated with water. Then 1 or 1,5 ml of the solution which is containing microorganisms is taken into a disposable cuvette. It is measured at 546 nm. The value is called as optical Density of microorganisms.

3.2 Techniques for Obtaining Pure Cultures

Drigalski spatula technique and 13-Strips technique are techniques for obtaining pure cultures. Living organisms which are in liquid medium are transferred into plates by Drigalski spatula technique.

In this methods, Plates, MM Solution,NB Solution, Drigalski spatula, Inoculating Loop are used as materials.

First, Drigalski spatula technique is used. In this technique, 100 µl of solution of microorganism is taken into MM plates by Eppendorf pipet. The Drigalski is immersed in ethanol, burned with flame and cooled. The bacteria suspension is rubbed over the whole MM plate. This procedure is also applied to the diluted solutions which occur same type of bacteria. Afterwards, the plates are put into the incubator with 30 °C for 7 days.

The plates are examined. Different type of bacteria species is selected. Selected species are diluted with 13 strips technique. By this way, pure strains are obtained.

3.3 Preparation of Plates and Its Solutions

Minimal Medium Solution and Plates are prepared as following:

20 g of KH_2PO_4 and 70 g of $Na_2HPO_4{:}2H_2O$ are dissolved in a 1000-mL volumetric flask and diluted to volume with water to obtain Buffer Solution, pH7.1 (PP20).100 g of $(NH_4)_2SO_4$, 20 g of $MgSO_4{:}7H_2O$, 1 g of Fe (III) NH_4-Citrat (28% Fe) and 100 ml of SE100 are dissolved in a 1000-ml volumetric flask and diluted to volume with distilled water to obtain SL100.

50 g of Ca $(NO_3)_2{:}4H_2O$ is dissolved in a 1000 ml volumetric flask and diluted to volume with distilled water to obtain Ca1000.

15 g of Agar-Agar is dissolved in a flask with 1000 ml distilled water, mixed and autoclaved at 121°C for 25 minutes. After the solution is cool enough, 50 ml of sterilized buffer solution (pH7.1, PP20), 10 ml of sterilized SL100 and 1 ml of sterilized Ca1000 are added into the flask under sterile condition and gently mixed. Then, about 15 ml of the solution is poured into each Petri plates. When plates are solidified, they are turned upside down and stored in their plastic bag.

Nutrient Broth Solution and Plates are prepared as following:

15 g of Agar-Agar and 8 g of Nutrient Broth are dissolved in a flask with 1000 ml distilled water, mixed and autoclaved at 121°C for 25 minutes. After the solution is cool enough, about 15 ml of the solution is poured into each Petri plates. When plates are solidified, they are turned upside down and stored in their plastic bag.

3 mmol Sodium Benzoate +MM Plates are prepared as following:

The only difference between normal MM and this type MM is that 0,4 g of C_6H_5COONa is added the Agar-Agar solution, mixed and autoclaved at 121°C for 25 minutes. Rest of the procedure is same as above.

0.9 % (w/v) Saline Solution are prepared as following:

9 g of NaCl is dissolved in a flask with 1000 ml of distilled water and mixed.

3.4 Isolation and Enrichment of BAL, BAD and NaB Degrading Bacteria Species

In this method, MM solution, MM plates, 0,9 % saline solution, 3 mmol of Benzoate +MM plates, Chemicals (BAL, BAD and NaB as substrate), Eppendorf caps, Shaker, Autoclave and Centrifuge are used as materials.

Different type of soil samples collected from a forest, sediment of a creek and around a gas station to isolate BAL, BAD and NaB degrading bacteria species.

Soil samples from the forest and sediment of a creek mixed well in a bucket. 100 ml of minimal medium solution was taken into three Erlenmeyer bottles which were autoclaved before. If other case was not indicated, all preparations and works were done in sterile conditions. Two of the Erlenmeyer bottles had evaporation chamber on their top and the third one had not. Small amount of well mixed soil mixture was added separately into three Erlenmeyer bottles. 15 µl of BAL and BAD as a substrate were also pipetted separately into each evaporation chamber. 0, 04 g of sodium benzoate as a substrate was dissolved in last Erlenmeyer bottle to make up 3 mmol of NaB solution. They were shaked with shaker which is in 30°C climate room at 100 rpm almost three weeks by adding appropriate substrates twice in a week. 1 ml of the solutions were taken from each Erlenmeyer bottle and put into new Erlenmeyer bottle which had freshly prepared 100 ml of minimal media as described above. They were again shaked with shaker which is in 30°C climate room at 100 rpm almost two weeks by adding appropriate substrates twice in a week.

To get pure BAL, BAD and NaB strains, minimal medium plates and 3 mmol Benzoate+MM plates were prepared before. From each Erlenmeyer bottle, diluted solutions were prepared as following:

To prepare diluted solutions of substrate specific microorganisms 100 µl of the stock solutions which was in each Erlenmeyer bottle was taken into Eppendorf-caps and 900 µl of 0, 9% saline solution was added into each Eppendorf-caps and mixed. By this way, (10^{-1}) dilution was obtained. This procedure was applied until obtaining (10^{-8}) dilutions.

From each dilution, two plates were prepared by pipeting 100 µl of the solutions into the plates. The procedure in Drigalski spatula technique was again applied. 15 µl of

substrate BAL, and BAD added separately into each MM-plate. Due to 3 mmol of Benzoate +MM plate had its substrate inside; adding substrate of NaB was not performed for this type plates.

Then, the plates were sealed with parafilms and incubated at 37°C for 24 to 48 hours. Each plate was inspected and different type strains were choosed. 13 strips technique was applied to the choosed strains to get pure species. When fungi growth was occurred in the plate, it was changed with another plate by using same procedure.

3.5 Determination of Number of Microorganisms

Pure strains in the stock solution were diluted as factor of 10^{-1}, 10^{-2}, 10^{-3}, 10^{-4}, 10^{-5}, 10^{-6}, 10^{-7}, and 10^{-8}. To determination of number of microorganisms, statistical procedures were applied.

In this method, Cultures, MM solution, MM plates, 0.9 % saline solution, 3 mmol of Benzoate +MM plates, Chemicals (BAL, BAD and NaB as substrate), Eppendorf caps, Shaker are used as materials.

To determine number of microorganism, diluted solutions of substrate specific micro organisms was prepared. 100 µl of the stock solutions which was in each Erlenmeyer bottle was taken into Eppendorf-caps and 900 µl of 0,9% saline solution was added into Eppendorf-caps and mixed. By this way, (10^{-1}) dilution was obtained. This procedure was applied until obtaining (10^{-8}) dilutions.

From each dilution, two plates were prepared by pipeting 100 µl of the solutions into the plates. The procedure in Drigalski spatula technique was again applied. 15 µl of substrate BAL, and BAD added separately into each MM-plate. Due to 3 mmol of Benzoate +MM plate had its substrate inside; adding substrate of NaB was not performed for this type plates. Then, the plates were sealed with parafilms and incubated at 37°C for 24 to 48 hours. Each plate was inspected and counted visually. Statistical procedures were applied.

3.6 Biochemical Short Tests

3.6.1 Oxidase test

The cytochrome oxidase oxidises the reduced cytochrome c. Through transfer of electrons to molecular oxygen, the reduced cytochrome oxidase is recovered. This enzyme reduces organic substance in presence of molecular oxygen. As an organic substance, indephenol blue is reduced by this enzymes whether bacteria has.
In this method, Cultures, Bactident Oxidase by Merck are used as materials.
With an inoculating loop, well grown colony was taken from MM plate and introduced to reaction area of the test strip. After 30-60 seconds the strip was compared with the colour scale.

In the case of cytochrome oxidase-positive germs the reaction zone is coloured blue to blue-violet. Bacteria without cytochrome oxidase are not adapted to O_2 and considered as anaerobic or facultative anaerobic.

3.6.2 Catalase test

Catalase is present in all cells which has aerobic metabolism. It degrades hydrogen peroxide into hydrogen and peroxide. It is used for determining taxonomic behaviour of bacteria.

In this method, Cultures, 3% aqueous solution of Hydrogen Peroxide are used as materials.With an inoculating loop, well grown colony was taken from MM plate and introduced to reaction area of the glass medium. A drop hydrogen peroxide solution is placed on the bacteria. If any reaction occurs, it is observed.

Positive reaction: Immediate gas formation (i.e. O_2) on the colony or bacteria mass means the examined strain is adapted to O_2.
Negative reaction: No gas development, which means no catalase enzyme is present.

3.7 Gram (-) Identification Test (BIOLOG Test)

The Biolog GN2 MicroPlate is designed for identification and characterization of a very wide range of aerobic gram (-) bacteria. It has redox chemistry. It based on reduction of tetrazolium, responds to the process of metabolism rather than metabolic by products. It performs 95 discrete tests simultaneously and gives a characteristic reaction pattern.

In this method, Cultures, GN2 MicroPlate and The MicroLog system software are used as materials.

A pure culture of a bacterium is grown on a Biolog Universal growth 5% sheep blood agar plate.

The bacteria are swabbed from the surface of the agar plate, and suspended to a specified density in GN/GP inoculating fluid.
150 µl of bacterial suspension is pipetted into each well of GN2 MicroPlate.
The MicroPlates is incubated at 30° or 35°C for 4-24 hours.
The MicroPlates are read visually and compared to GN Database by the software.

3.8 Identification of Bacteria by API 20 NE

The API-20-NE test is designed for identification and characterization of an aerobic gram (-) bacteria. It can not identify *Escherichia coli* and *Enterobacter sp.* It performs 20 discrete tests simultaneously and gives a characteristic reaction pattern.

In this method, Cultures, API 20 NE incubation chamber, AUX ampule and The Database system are used as materials.

A pure culture of a bacterium is grown on a MM plate. API 20 NE Chamber is humidified by 1 ml water. The bacteria are swabbed from the surface of the agar plate, and suspended to inoculating fluids. 100 µl of bacterial suspension is pipetted into each well of API 20 NE Chamber. The Chamber is incubated at 30° for 48-72 hours. The kit are read visually and compared to Database.

3.9 Growth and Biodegradation Curves

In this method, Cultures, Buffer Solution, pH7.1 (PP20), SL100, Ca1000, Spectrophotometer Mobile phase and HPLC are used as materials. Substrates (i.e. BAL and BAD) are used as carbon and energy source by micro organisms. As degradation proceeds, the number of microbial cells or the biomass increases. The cell growth is measured by measurement of OD at 546 nm. Degradation rate (i.e. degradation kinetics) is the relative percentage of degradation referred to the starting concentration of the substrate. Since the microorganisms use substrate as carbon and energy resource, as the degradation proceeds, biomass increases and substrate

decreases. By plotting substrate concentration vs. time of incubation (in 30 minutes intervals), we can obtain kinetic information about the degradation process. Tests are performed by species of BAL 100, 110, 120, 200, 310, and that of BAD 400, 900, 910, 920, 930 and 950. In the former degradation tests, species of BAL 200, 215 and that of BAD 900, 930 which had the highest optical density after one week incubation showed a very good performance in degrading BAL and BAD substrate. BAL and BAD concentration in the liquid cultures were determined by HPLC. Their OD's were determined by Spectrophotometer.

20 ml buffer solution, pH7.1 was taken into a flask with volumetric pipette and flask was autoclaved at 121°C for 25 minutes. After the flask cooled down, 200 µl of sterile SL100 and 20 µl of sterile Ca1000 were added into the flask with pipette. A separate, well grown colony was taken from the culture medium into the flask by inoculating loop and according to substrate solubility value various amount of substrate (0, 01, 0, 1, 0, 25, 0, 5, 0, 75, 1, 0 of g / L BAL and BAD) were added separately into the flask. Then, the flask was shaked with the shaker at ~95 rpm and in 30°C. The initial optical density of this liquid culture was determined at 546 nm by the spectrophotometer and initial BAL and BAD concentration was determined by HPLC. Then, the flask was shaked again with the shaker at ~95 rpm and in 30°C. The OD_{546} and BAL, BAD concentration tests are performed in half hours intervals until their concentration decreases to zero.

3.10 HPLC Calibration Curves

51 µl of BAD are dissolved in a 100-mL volumetric flask and diluted to volume with distilled water and mixed well to prepare 5 mmol / L of BAD stock solution. 52 µl of BAD are dissolved in a 100-mL volumetric flask and diluted to volume with distilled water and mixed well to prepare 5 mmol / L of BAL stock solution. 0, 072 g of BAD are dissolved in a 100-mL volumetric flask and diluted to volume with distilled water and mixed well to prepare 5 mmol / L of NaB stock solution. In this method, HPLC and Mobile phase are used as materials.

Analyse Conditions:

Detector wavelength	: 210 nm
Column	: Prontosil Eurobond
Column temperature	: Ambient

Injection volume : 20 µl
Flow rate : 1 ml/min
Analyse time : 15 minutes
Mobile Phase : It contains 500 ml of methanol, 1500 ml of distilled water and 2 ml of ortho-Phosphoric acid 85 %. The air bubbles were removed by using a vacuum pump

Further dilutions were prepared with 5 mmol / L of BAD, BAL and NaB stock solutions as following (i.e. from 1 mmol / L to 0,1 mmo l/ L);

0, 4, 0, 36, 0, 32, 0, 28, 0, 24, 0, 20, 0, 16, 0, 12, 0, 08, and 0, 04 ml of each stock solution was taken into HPLC vials. They were diluted to 2 ml with distilled water. These samples were analyzed with the HPLC considering the analyse conditions above. Then, calibration curves were obtained as absorption vs. concentration.

3.11 Preparation of Pure Liquid Cultures for BAL, BAD Degrading Bacteria Species

In this method, MM solution, MM plates, 0,9 % saline solution, 3 mmol of Benzoate +MM plates, Chemicals (BAL, BAD and NaB as substrate), Eppendorf caps, Shaker, Autoclave and Centrifuge are used as materials.

To get pure strain solution, a separate, well grown colony was taken from the plate which was prepared with 13 strips technique by inoculation loop and inoculated into freshly prepared 1 L of MM solution. They were shaked with shaker which is in 30°C climate room at 100 rpm almost two days by adding 150 µl of appropriate substrates. These solutions after decreasing their volume by centrifuge were used to inoculate the biofilter reactor later.

3.12. Generation and Metabolism Rate Tests

In this method, MM solution, Chemicals (BAL, BAD as substrate), Cultures, spectrophotometer, Mobile phase and HPLC are used as materials.

In this experiment kinetics of the BAL and BAD metabolism is to be determined by the respective bacteria species. To determine metabolism rate calorimetric procedure is used. The principle is the same like in 4.10 Growth and degradation curve. The results

of the OD_{546}-Measurement are plotted vs. time. The maximum growth rate μ is to be determined as:

t_1 presents starting time of growth at which OD value is presented as c_1.
t_2 presents stopping time of growth at which OD value is presented as c_2.

$$\mu = \ln (c_2-c_1) / (t_2-t_1), [h^{-1}]$$

Generation time, t_d

$$t_d = \ln 2 / \mu, [h]$$

The metabolism rate (i.e degradation rate), r is to be determined as;

$$r=(c_1-c_2) / [(t_1-t_2)*(OD_1-OD_2) / 2], [mmol/(L.h.OD)]$$

c_1, and c_2 : concentration, [mmol/L]
OD_1, and OD_2 : optical density, [-]
t_1, and t_2 : time, [h]

Procedure is same that of 4.10 Growth and degradation curve.

3.13 Simulation of Lab-Scale Biofilter

There are three types of bioreactors for waste gas purification. These are biofilter, biotrickling filter and bioscrubber. They differ in the location of microorganisms present and the flow of water phase. In this study, the biofilter was used to purify waste air (Figure 3.1, 3.2, 3.3, 3.4 and 3.5). In this type of bioreactor, microorganisms stay on filter material as biofilm. Water was circulated through the lab-scale waste air purification reactor.

Values for reactor:

Height : 50 cm
Diameter : 10 cm
Volume : 0,017 m^3
Crude air flow : 0, 285, 0, 493, 0, 667 m^3/h

Due to need and installation of different air pumps, we get three different air flows.

Figure 3.1: A view of biofilter reactor.

Its height and diameter is 50 cm.and 10 cm respectively. Its volume is 0,017 m^3. The values of crude air flow are 0, 285, 0, 493, 0, 667 m^3/h

Figure 3.2: Water cycling pump

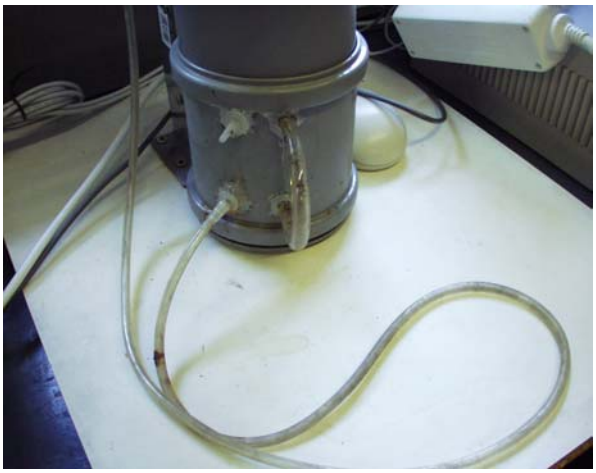

Figure 3.3: Water levelling pipe, Water sampling outlet, Air entrance hole.

Figure 3.4: Water shower

Figure 3.5: Wood particles

Fertilizer concentration is 0,0004 ml/ml water. Its density is 3,25kg/2,5L (i.e. 1,3 kg/L). Fertilizer solvent has 8 % of total Nitrogen, 4,5% of water soluble Phosphate, 10,8 % of water soluble Calcium, 10 ppm of water soluble Boron, 2 ppm of water soluble copper, 20 ppm of water soluble Iron, 10 ppm of water soluble Manganese, 10 ppm of water soluble molybdenum and 20 ppm of water soluble Zinc. Content conditioning agent is EDTA

4. RESULTS AND DISCUSSION

Simulating and monitoring of the system is main duty of this study. To achieve this aim, isolated species were inoculated into the system. The species were examined by classical microbiological tests. Their growth and degradation abilities were compared. Moreover, their behaviours in the system were observed (i.e. pH change, temperature effect, efficiency, tolerance to gas flow amount, load, and specific degradation rate and substrate amount in the eluent).

Observed results indicate that main problem in the system is sudden acidification to pH 4.5. This leads to the death of the bacteria. Additional problems are the accumulation of benzyl alcohol up to the its saturation limit in water and naturally corrosion of the biofilter. Beyond this point the smell is another problem. The most important problem is the effects of benzyl alcohol onto the set ups of the biofilter.

4.1 Isolation and Enrichment of BAL, BAD and NaB Degrading Bacteria Species

Strains which obtained from forest, sediment of a creek and around a gas station are BAL 100, 110, 120, 200, 215, 300, 310, 320, 400, 500, 600, 700, 800 and BAD 100, 200, 300, 400, 500, 600, 900, 910, 920, 930, 940, and 950. No strains developed on NaB plates. The study should be redoned for NaB Strains.

4.2 Determination of Number of Microorganisms

Comparing to their growth and metabolism rate, BAL 200 and 215, BAD 900 and 930 species are selected as benzyl alcohol degrading species (Table 4.1).

Table 4.1: The OD_{546} and cell number correlations of BAL 200 species.

Dilution Degree	*NB*		*MM + BAL (as substrate)*	
		Calculated		*Calculated*
0		N/A		N/A
10^{-5}	284	$2,84 \times 10^{8}$	292	$2,92 \times 10^{8}$
10^{-6}	43	$4,3 \times 10^{8}$	26	$2,6 \times 10^{8}$
10^{-7}	12	$1,2 \times 10^{9}$	6	6×10^{8}
Cell number / ml		$6,4 \times 10^{8}$		$3,8 \times 10^{8}$
Cell number / ml OD		$2,84 \times 10^{8}$		$4,8 \times 10^{8}$

The OD_{546} of species of BAL 200 was 0,796.

BAL 200 species are inoculated onto NB and MM plates in different dilution concentrations. Benzyl alcohol is used as the substrate. Their cell number per ml and OD was calculated.

BAL 215 species are inoculated onto NB and MM plates in different dilution concentrations. Benzyl alcohol is used as the substrate. Their cell number per ml and OD was calculated (Table 4.2).

Table 4.2: The OD_{546} and cell number correlations of BAL 215 species.

Dilution Degree	*NB*		*MM + BAL (as substrate)*	
		Calculated		*Calculated*
0		N/A		N/A
10^{-5}	N/A	N/A	204	$2,04 \times 10^{8}$
10^{-6}	132	$1,32 \times 10^{9}$	44	$4,4 \times 10^{8}$
10^{-7}	42	$4,2 \times 10^{9}$	17	$1,7 \times 10^{9}$
Cell number / ml		$2,76 \times 10^{9}$		$7,8 \times 10^{8}$
Cell number / ml OD		$1,6 \times 10^{9}$		$4,6 \times 10^{8}$

The OD_{546} of species of BAL 215 was 1,704.

This study was not achieved for BAD 900 species due to the experimental failure.

BAD 930 species are inoculated onto NB and MM plates in different dilution concentrations. Benzyl alcohol is used as the substrate. Their cell number per ml and OD was calculated (Table 4.3) (Figure 4.1). These experiments show that BAD species number is more than BAL species.

Table 4.3: The OD_{546} and cell number correlations of BAD 930 species.

Dilution Degree	*NB*		*MM + BAL (as substrate)*	
		Calculated		*Calculated*
0		N/A		N/A
10^{-5}	N/A	N/A	208	$2,08 \times 10^{8}$
10^{-6}	82	$8,2 \times 10^{8}$	38	$3,8 \times 10^{8}$
10^{-7}	72	$7,2 \times 10^{9}$	34	$3,4 \times 10^{9}$
Cell number / ml		$4,01 \times 10^{9}$		$1,33 \times 10^{9}$
Cell number / ml OD		$4,0 \times 10^{9}$		$1,3 \times 10^{9}$

The OD_{546} of species of BAD 930 was 0,992.

4.3 Biochemical Short Tests (Taxonomy)

Oxidase Test

The species of BAL 200 is oxidase positive.

The species of BAD 900 is oxidase positive.

The species of BAD 930 is oxidase positive

They are adapted to O_2 and considered as aerobic.

Catalase Test

The species of BAL 200 is catalase positive.

The species of BAD 900 is catalase positive.

The species of BAD 930 is catalase positive.

They are adapted to O_2 and considered as aerobic.

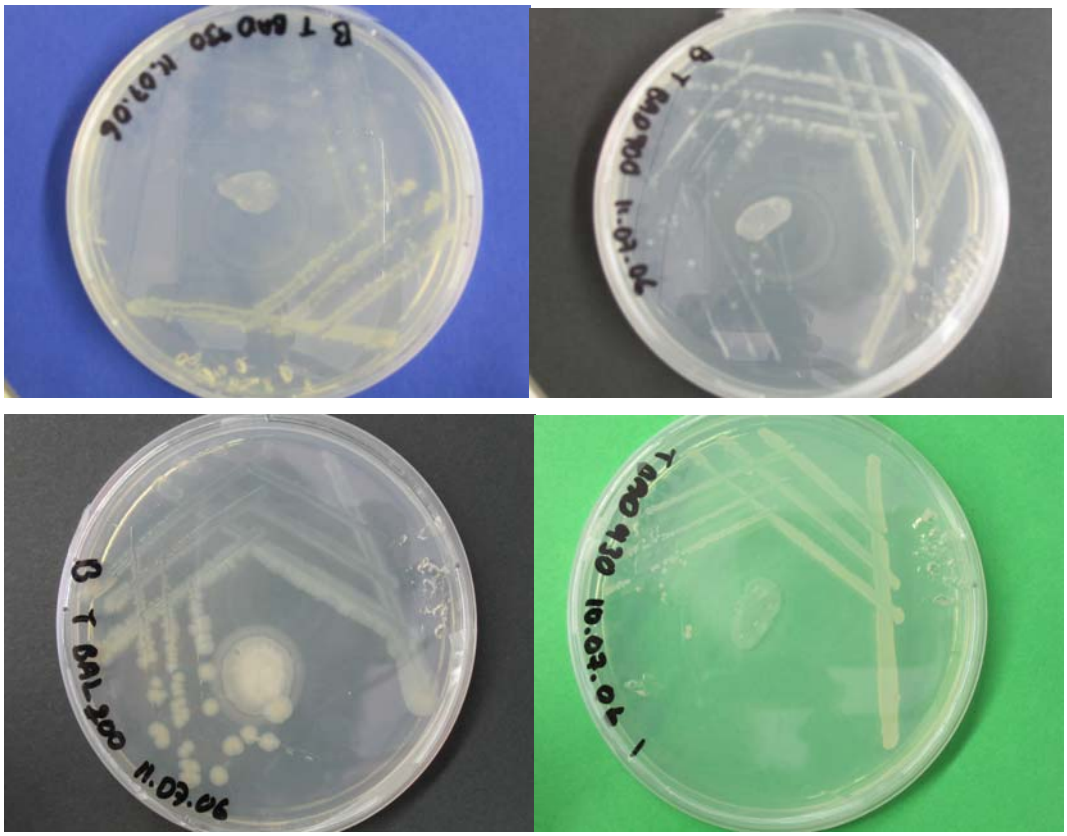

Figure 4.1: Photos of some plates of Species BAL 200, BAD 900 and 930

4.4 Gram Negative Identification Test (BIOLOG)

The species of BAL 200 is "*Acinetobacter calcoaceticus/genospecies 3*" (90%)

Description: An aerobic gram-negative bacillus widely distributed in nature (soil, sewage, and water) and in the hospital environment. It is able to survive on both moist and dry surfaces and may be part of the normal skin flora of humans. It causes hospital-acquired respiratory, urinary tract, wound infections, abscesses, and meningitis in debilitated humans.

The species of BAL 215 is "*Acinetobacter calcoaceticus/genospecies 3*" (99%) *"A.baumanii/genospecies2"* (87%).

A.baumanii/genospecies2

Description: *Acinetobacter baumannii* is an opportunistic pathogen operating in hospitals creating serious infections such as pneumonia. It principally affects patients who have weakened health and this is why we call it opportunistic. Moreover, the

mortality rate from these infections are usually high given, on the one hand, the weakness of the patient and, on the other, *A. baumannii* is resistant to many antibiotics. Furthermore, once a specific course of treatment is prescribed for *A. Baumannii*, the pathogen has a great capacity for acquiring resistance to these antibiotics.

The species of BAD 900 is "*Pseudomonas putida biotype A* " (99%)

Description: An aerobic, oxidase-positive, gram-negative bacillus that produces a yellowish colony. This isolate of *Pseudomonas putida* has been identified as belonging to biotype A using the Biolog classification system. It can be isolated from soil, water, plants, and foodstuff, including milk. The majority of strains are nonpathogenic, but some strains may rarely be opportunist pathogens.

The species of BAD 930 is "*Ralstonia paucula (Cupeiavidus pauculus)* "(82%)*"Acinetobacter genospecies 14"* (61%).

Description: Bacterium now known as *Burkholderia cepacia*. ***Burkholderia cepacia*****:** (*B. cepacia*, for short.) A group of bacteria found in soil and water that are often resistant to common antibiotics. *B. cepacia* poses little medical risk to healthy people. However, people who have certain health problems such as a weakened immune systems or chronic lung disease, particularly cystic fibrosis (CF), are more susceptible to infection with it. Transmission of *B. cepacia* has been reported from contaminated solutions and medical devices as well as contaminated over-the-counter nasal spray and contaminated mouthwash. *B. cepacia* can also be spread to susceptible persons by person-to-person contact, contact with contaminated surfaces, and exposure to *B. cepacia* in the environment. Careful attention to infection control procedures like hand hygiene can help reduce the risk of transmission of this organism. Decisions on the treatment of infections with *B. cepacia* are best made on a case-by-case basis. Usually it can be treated with drugs called Trimethoprim-sulfamethoxazole or Meropenem.

Acinetobacter genospecies 14

Description: Gram negative aerobic bacteria. These organisms are included in the dangerous pathogens supplement.

According to the descriptions, when performing experiments, species should be handled with care.

Table 4.4. shows the identification of Bacteria by API 20 NE Test.

Table 4.4: Identification of Bacteria by API 20 NE Test.

	After 24 h	After 48 h
BAL 200	*Pseudomonas cepacia* (4%)	*Pseudomonas cepacia* (99.9%)
BAL 215	*Pseudomonas cepacia* (4%)	*Pseudomonas cepacia* (99.9%)

	After 24 h	After 48 h
BAD 900	*Pseudomonas cepacia* (4%)	*Pseudomonas cepacia* (99.8%)
BAD 930	*Pseudomonas cepacia* (99%)	*Pseudomonas cepacia* (99%)

4.5 HPLC Calibration Curves of the Substrates

HPLC Calibration Curves of the Substrates are illustrated in Figure 4.2, 4.3 and 4.4)

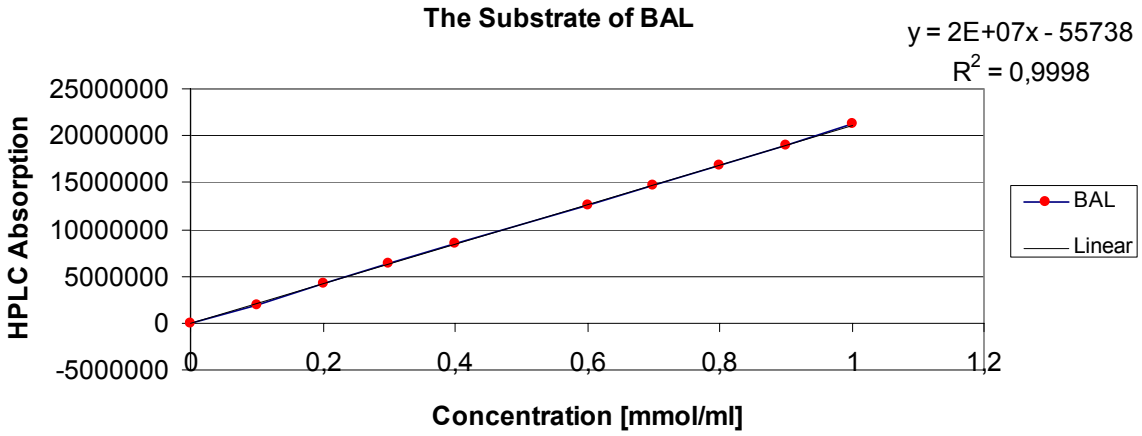

Figure 4.2: HPLC calibration curve of the substrate of BAL.

y describe its absorption and x describe its concentration in mmol / ml. Alpha numeric a is intersection point, b is slope of the line, r is linear regression coefficient in its linear formula.

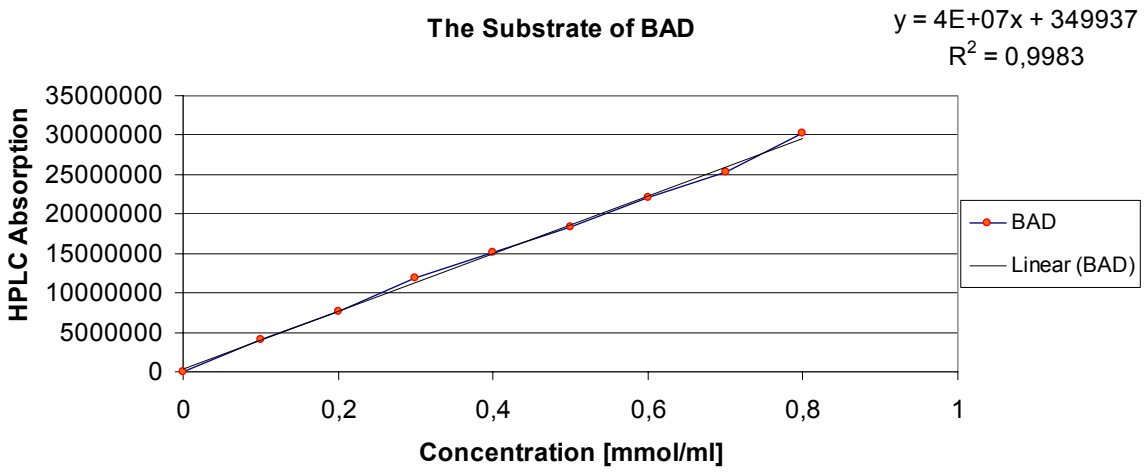

Figure 4.3: HPLC calibration curve of the substrate of BAD.

y describe its absorption and x describe its concentration in mmol / ml. Alpha numeric a is intersection point, b is slope of the line, r is linear regression coefficient in its linear formula.

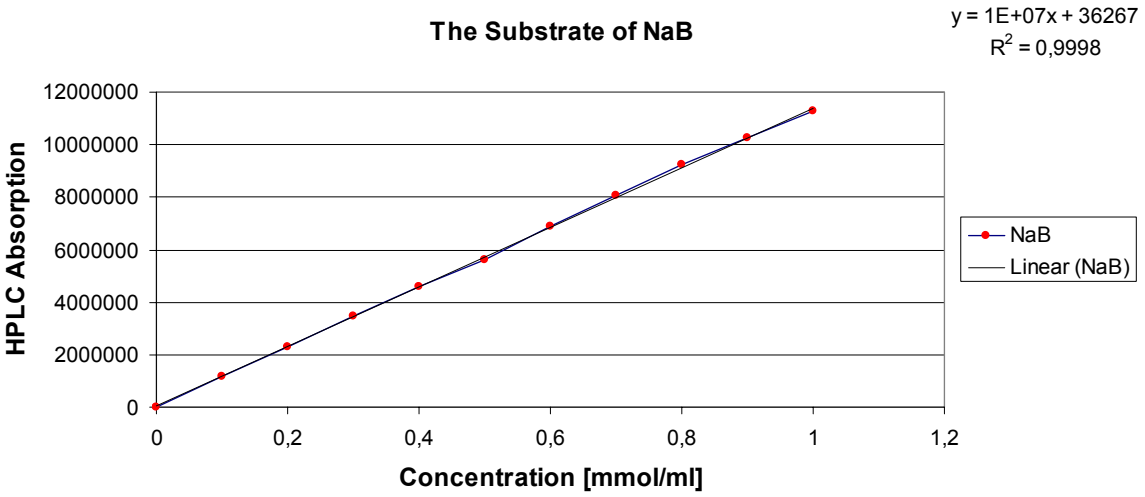

Figure 4.4: HPLC calibration curve of the substrate of NaB.

y describe its absorption and x describe its concentration in mmol / ml. Alpha numeric a is intersection point, b is slope of the line, r is linear regression coefficient in its linear formula.

4.6 BAL Growth & Degradation Tests

The purpose of this experiment is that Different BAL Species is compared to their growth rate using the same substrate. By this way, the fastest species are selected to inoculate into bioreactor. Figure 4.5 shows the growth curves of different BAL species using the same substrate (2mmol/L BAL).

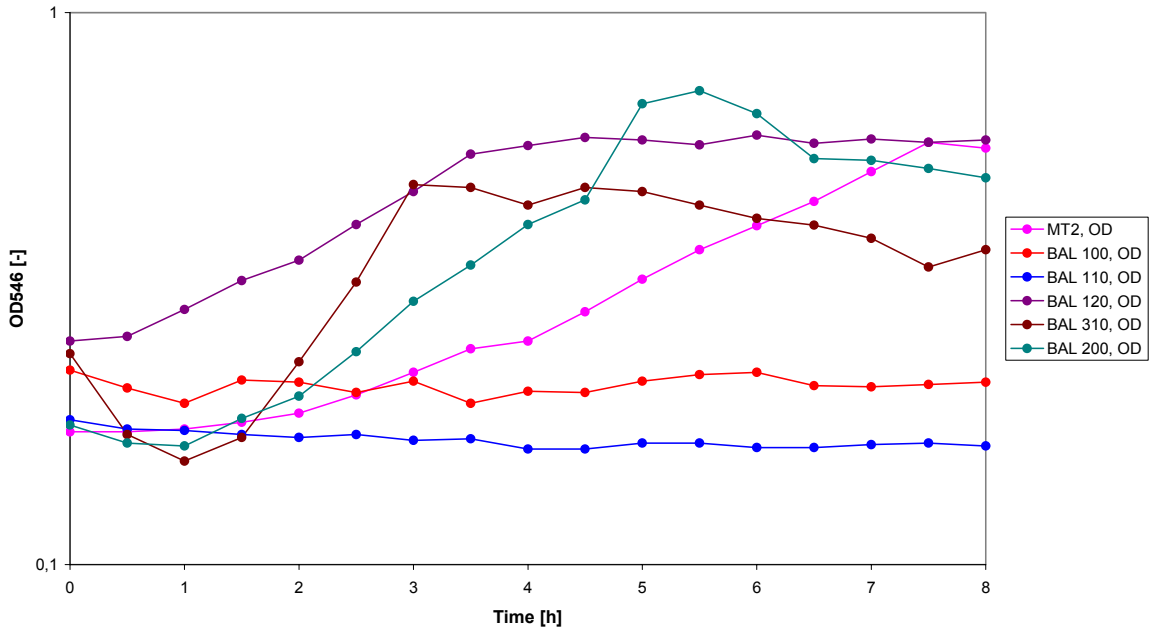

Figure 4.5: Growth curves of different BAL species using the same substrate (2mmol/L BAL).

BAL 200 species is the one of the best of this study. It is choosed for further studies. MT2 species is well known DNA modified reference species. BAL 100 and BAL 110 are less performance species in this study. Figure 4.6. gives the substrate (2 mmol / L BAL) degradation curves of different BAL species.

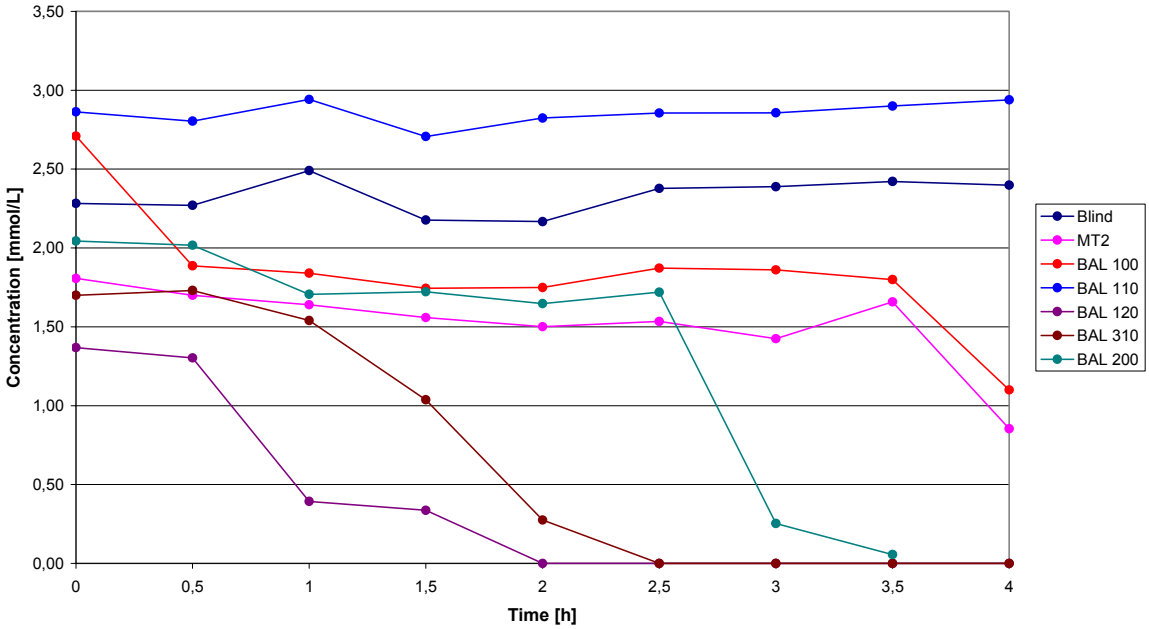

Figure 4.6: Substrate (2 mmol / L BAL) degradation curves of different BAL species

The degradation performance of each BAL species compared. BAL 200 species also performed well in this test as in the growth test. So, it is choosed for furder studies. It degrade 2 mmol / L Benzyl alcohol in 3,5 hours. Growth of Species BAL 200 are shown in Figure 4.7.

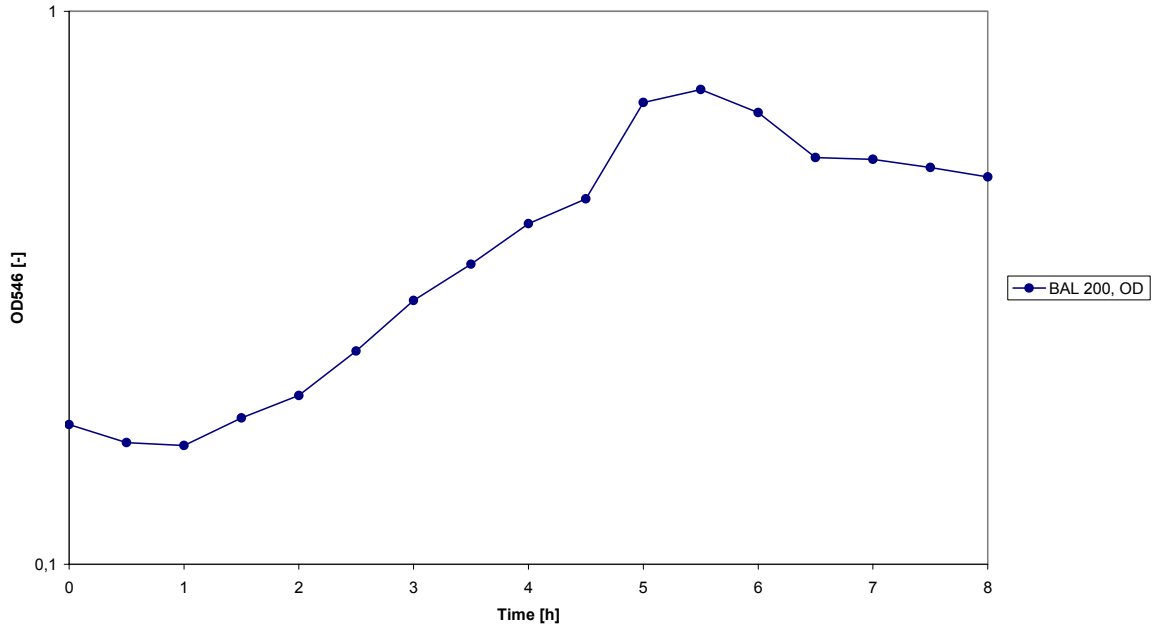

Figure 4.7: Growth of Species BAL 200

It is selected among the other BAL species due to its growth rate. It reaches its plateau value at 5 hours.

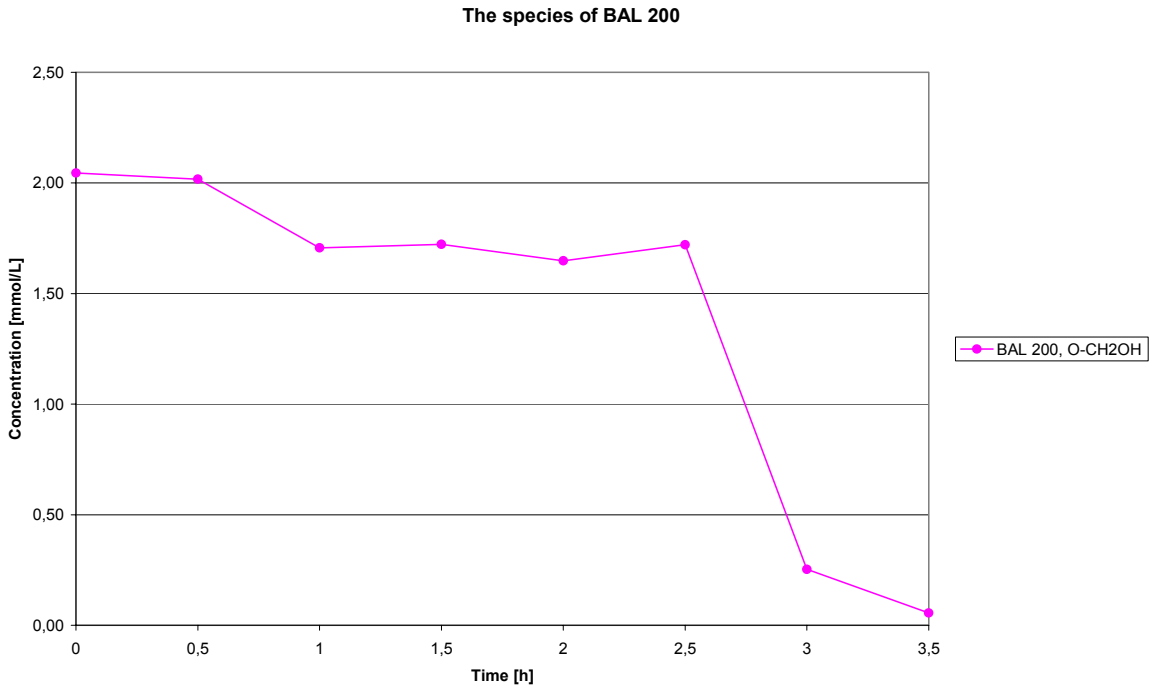

Figure 4.8: Degradation curve of Species BAL 200

It is selected among the other BAL species due to degradation ability of 2 mmol / L of substrate BAL in 3,5 hours (Figure 4.8).

4.7 BAD Growth and Degradation Tests

Different BAD Species is compared to their growth rate using the same substrate. By this way, the fastest species are selected to inoculate into bioreactor. According to graph BAD 900 and Bad 930 species have good performance in growth ability (Figure 4.9). Due to this fact, they choosed for further studies.

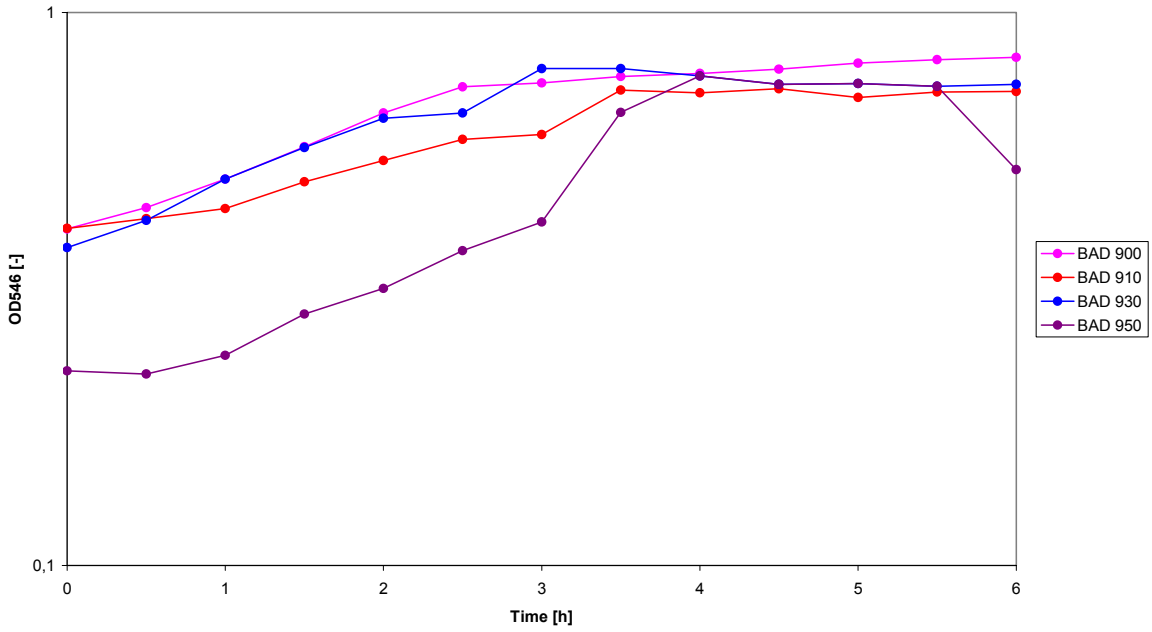

Figure 4.9: Growth curves of different BAD species using the same substrate (2 mmol / L Benzyl aldehyde).

It is selected among the other BAD species due to its growth rate in 2 mmol / L substrate Benzyl aldehyde (Figure 4.10). It reach plateau value at 3 hours.

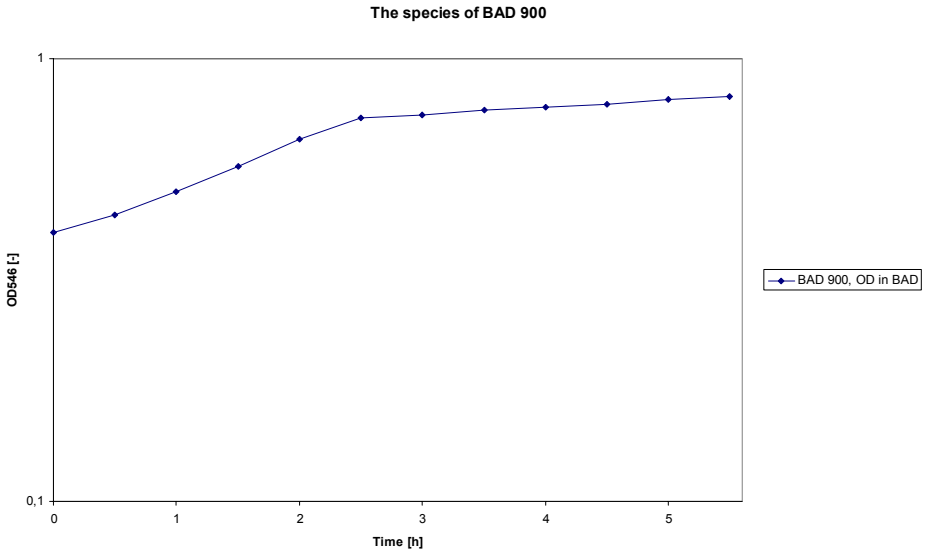

Figure 4.10: Growth curve of Species BAD 900

It is selected among the other BAD species due to degradation ability of 2 mmol / L (Figure 4.11). Benzyl aldehyde. It reached 0 value almost 1, 5 hours.

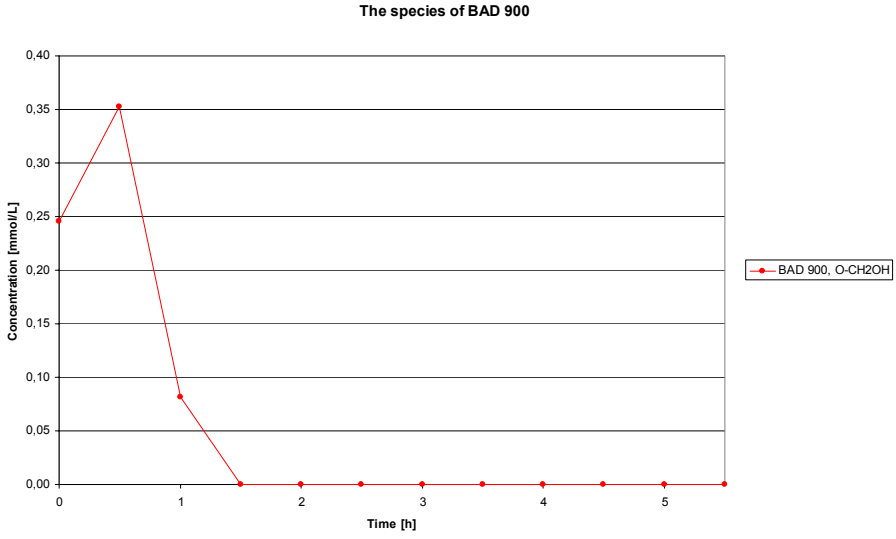

Figure 4.11: Degradation curve of Species BAD 900

It is selected among the other BAD species due to its growth rate.Its OD_{546} values are obtained in 2 mmol / L of BAD substrate (Figure 4.12). It reaches its plateau value at 3 hours

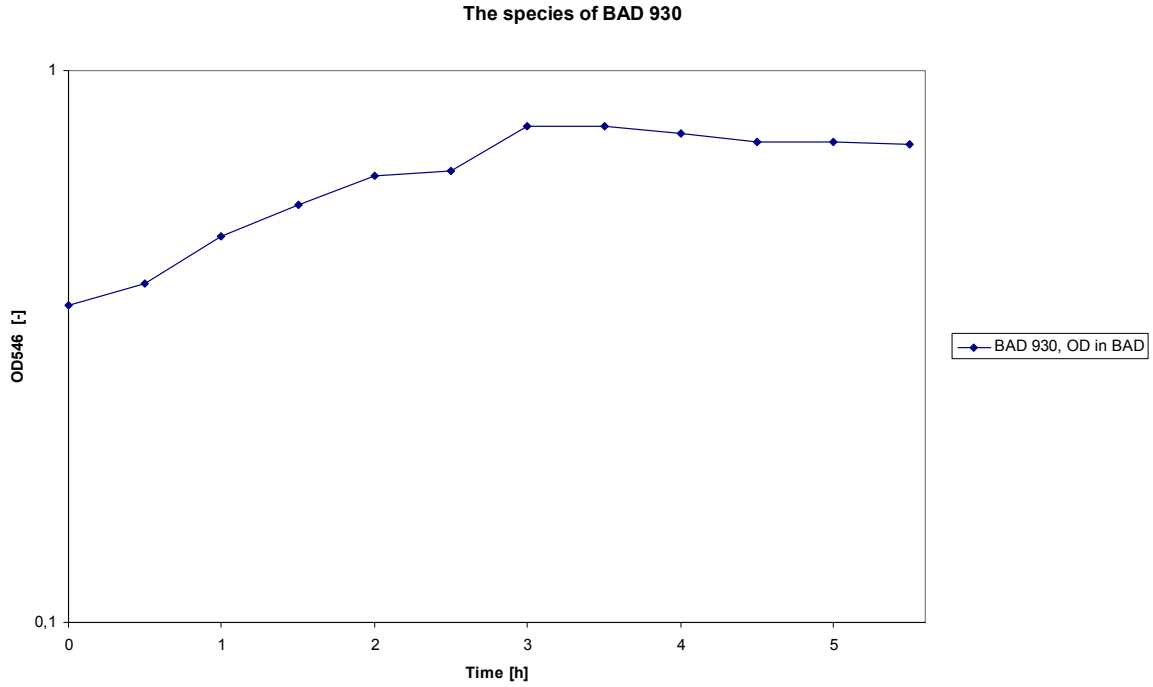

Figure 4.12: Growth curve of Species of BAD 930

It is selected among the other BAD species due to degradation ability of substrate Benzyl aldehyde (Figure 4.13). It degrade 2 mmol / L Benzyl aldehyde in 1 hours .

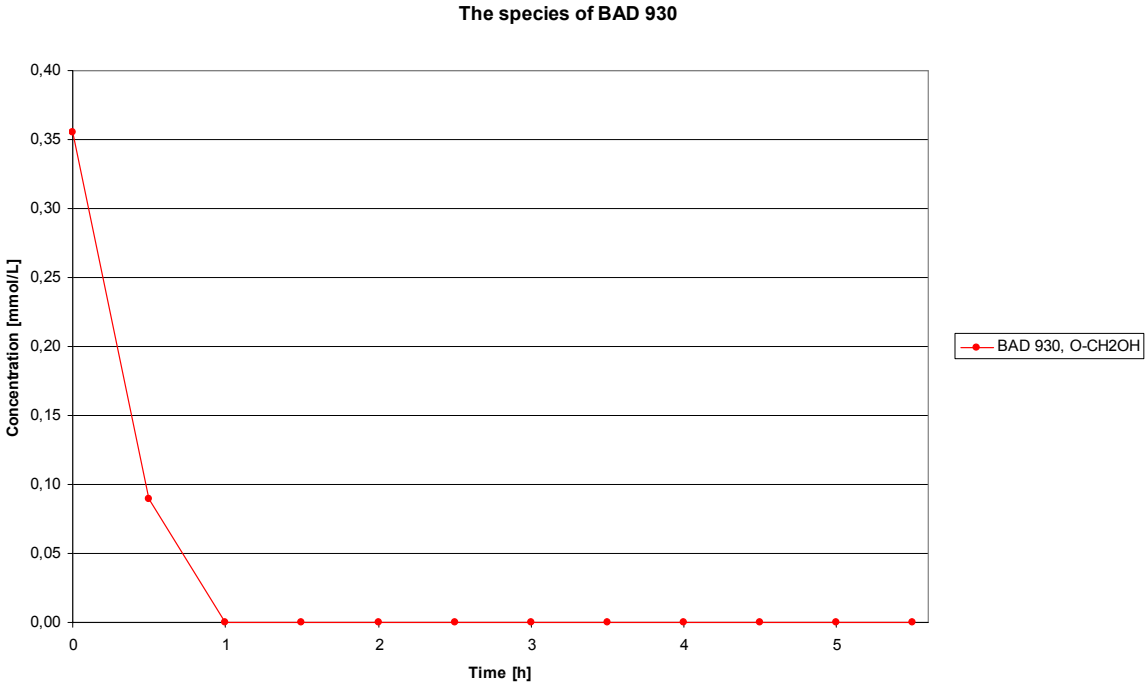

Figure 4.13: Degradation curve of Species of BAD 930

Growth characteries of BAL and BAD Species on benzoate substrate is shown in Figure 4.14. It is obtained using 2 mmol/L NaB. By this way, their ability to growth in NaB is measured.

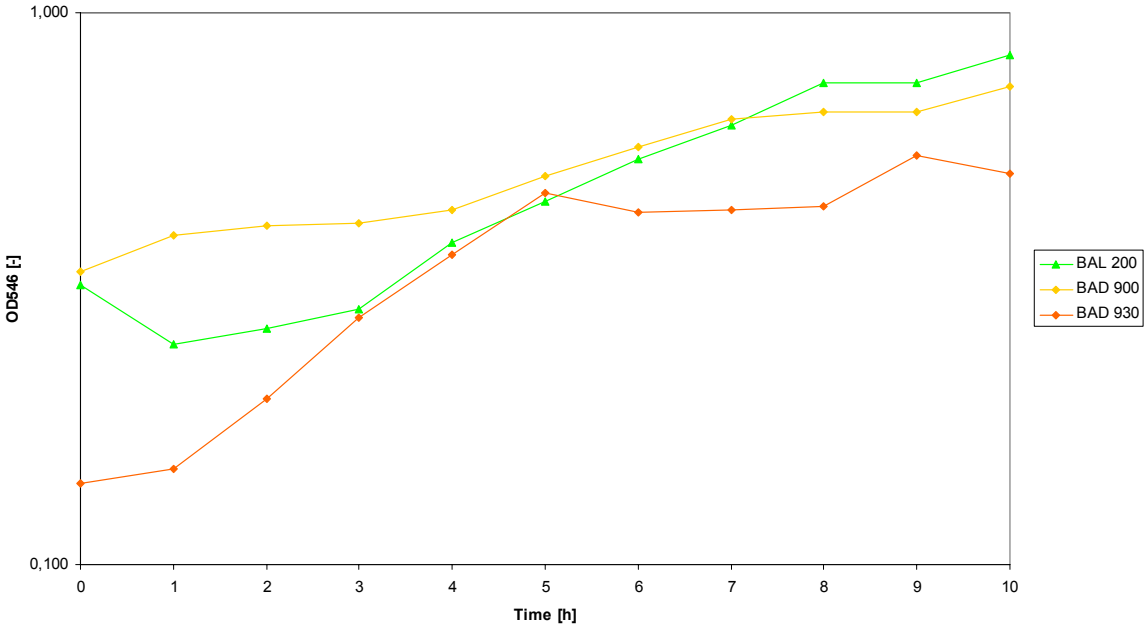

Figure 4.14: Growth curves of Species of BAL 200, BAD 900 and 930 on Benzoate.

4.8 Generation and Metabolism Rate tests

Growth rate constant and generation time together with metabolism rate are given in Table 4.5.

Table 4.5: Growth rate constant and generation time values of BAL and BAD degrading species.

	Start-OD_{546}	*End-OD_{546}*	*t_0*	*t*	*dt*	*μ [h^{-1}]*	*td [h^{-1}]*
BAL200	0,202	0,684	2	5	3	0,41	1,7
BAD900	0,406	0,746	0	3	3	0,20	3,4
BAD930	0,376	0,792	0	3	3	0,25	2,8

Table 4.6: Metabolism rate (i.e. degradation rate) values of BAL and BAD degrading species.

	Start-OD_{546}	*End-OD_{546}*	*t_0*	*t*	*dt*	*dc*	*r [mmol/LhOD]*
BAL200	0,243	0,413	2,5	4	1,5	1,72	3,5
BAD900	0,406	0,746	0	3	3	1,24	0,72
BAD930	0,376	0,792	0	3	3	1,57	0,9

The average BAL metabolism rate of BAL 200 is 3.5 mmol/(LhOD), 378 mg / (LhOD)
The average BAD metabolism rate of BAD 900 is 0,72 mmol/(LhOD), 76 mg / (LhOD)
The average BAD metabolism rate of BAD 930 is 0,9 mmol/(LhOD), 95 mg / (LhOD)

4.9. Performance of the Reactor

HPLC results of reactor eluent and performance criteria of biorector.are illustrated in Table 4.7 and Table 4.8.

Table 4.7: HPLC Absorption and concentration values of ingredients (BAL, BAD and NaB) in eluent of Bioreactor.

	Concentrations of Substrates in Eluent			
Date	Number of days	g BAL/L	g BAD/L	g NaB/L
29. Sep	1	0	0	0
30. Sep	2	0,9	0	0
03. Okt	5	3	0	0,8
04. Okt	6	0	0	0
05. Okt	7	4		0,9
06. Okt	8	4		0,8
07. Okt	9	5		0,9
10. Okt	12	8		1,1
11. Okt	13	8		1,2
12. Okt	14	7		1,3
14. Okt	16	10	0,03	1,8
17. Okt	19	11	0,03	2,1
18. Okt	20	11	0,03	2
20. Okt	22	12	0,03	2,1
21. Okt	23	11	0,03	1,9
24. Okt	26	14		2,8
24. Okt	26	14		2,9

Table 4.8: Performance criteria of Biorector.

Date	*pH*	*Temp, ˚C*	*Vol Filter Material V_{fs}, m^3*	*efficiency n, %*	*crude gas ρ_{rol}, mgC/m^3*	*crude gas ρ_{rol}, $mgBAL/m^3$*	*pure gas ρ_{rein} mgC/m^3*	*pure gas ρ_{rein}, $mgBAL/m^3$*	*gas volume v^h, m^3/h*	*vol specific gas flow v_v^h, $m^3/h\ m^3$*	*Load $mgorgC/m^3\ h$*	*specific degradation rate E_v, $mgorgC/m^3\ h$*
04.10			0,017	13,9	252,9	325,2	217,8	280,0	0,285	16,8	4240,3	588,9
05.10	5,0	25,7	0,017	59,1	309,1	397,5	126,5	162,6	0,285	16,8	5182,5	3062,4
06.10	5,0	25,3	0,017	87,8	288,1	370,4	35,1	45,2	0,285	16,8	4829,2	4240,3
07.10	4,9	24	0,017	99	281,0	361,3	2,8	3,6	0,285	16,8	4711,4	4664,3
10.10	5,0	24,9	0,017	45,1	288,1	370,4	158,1	203,2	0,285	16,8	4829,2	2179,0
11.10	5,0	23,3	0,017	42,1	266,9	343,3	154,6	198,7	0,285	16,8	4475,8	1884,6
11.10	7,3	24,2	0,017	46,5	302,1	388,4	161,6	207,8	0,493	29,0	8764,7	4076,6
12.10	7,2	21,7	0,017	51,4	259,9	334,2	126,5	162,6	0,493	29,0	7541,7	3872,8
14.10	7,1	23,1	0,017	47,6	288,1	370,4	151,1	194,2	0,493	29,0	8357,0	3974,7
17.10	7,1	21,6	0,017	75,8	231,9	298,1	56,2	72,3	0,493	29,0	6726,4	5095,8
18.10	7,1	22	0,017	42,5	281,0	361,3	161,6	207,8	0,493	29,0	8153,2	3465,1
20.10	7,0	24	0,017	44,2	365,3	469,7	203,7	261,9	0,493	29,0	10599,2	4688,1
21.10	6,9	24,6	0,017	42,3	365,3	469,7	210,8	270,9	0,493	29,0	10599,2	4484,3
25.10			0,017	87,4	333,7	429,1	42,2	54,2	0,493	29,0	9681,9	8458,9
02.10	6,4	21,6	0,017	33,1	727,2	934,9	486,4	625,4	0,493	29,0	21096,4	6985,1
07.10	6,1	21,8	0,017	24,7	632,3	812,9	476,3	612,3	0,493	29,0	18344,7	4527,4
11.10			0,017	41,5	145,4	186,9	85,1	109,4	0,667	39,2	5706,1	2366,4
15.10	6,5		0,017	97,5	2533,3	3257,1	63,2	81,3	0,667	39,2	99396,1	96915,2

Formulary used in creating table 4.8 is as follows.

Vol Filter Material V_{fs}, [m^3] $V_{fs}=\Pi*r^2*h$ (Volume of the filter material in cylindrical tube. r: the radius of cylinder, h: the height of cylinder)

efficiency n, [%]

[(crude gas ρ_{rol}, [mgBAL/m^3] - pure gas ρ_{rein}, [mgBAL/m^3]) / crude gas ρ_{rol}, [mgBAL/m^3]]*100

crude gas ρ_{rol}, [mgC/m^3]

crude gas ρ_{rol}, [mgBAL/m^3]

pure gas ρ_{rein}, [mgC/m^3]

pure gas ρ_{rein}, [mgBAL/m^3]

gas volume v^h, [m^3/h]

vol specific gas flow v_v^h, [m^3/(h-m^3)]

(gas volume v^h, [m^3/h])/ (Vol of Filter Material V_{fs}, [m^3])

Load, [mgorgC/h] (crude gas ρ_{rol}, [mgC/m^3])* (vol specific gas flow v_v^h, [m^3/(h-m^3)]

specific degradation rate E_v, [mgorgC/(m^3-h)]

[(crude gas ρ_{rol}, [mgC/m^3])-(pure gas ρ_{rein}, [mgC/m^3])* (vol specific gas flow v_v^h, [m^3/(h-m^3)])]

Graphics of reactor performance for daily pH values is given in Figure 4.15. Daily crude and pure gas values of bioreactor is also given in Figure 4.16.

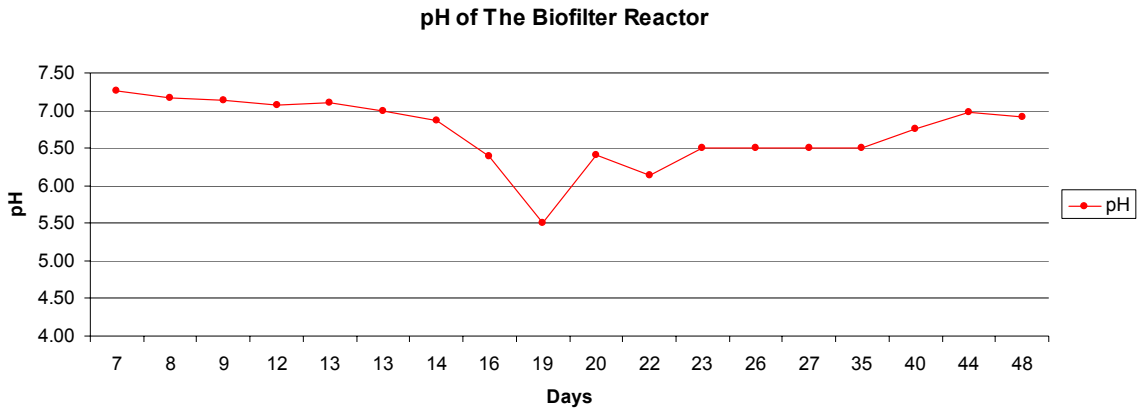

Figure 4.15: Daily pH values of Bioreactor

These graphic shows us after the inoculation of BAL degrading species, there is sudden decrease of pH value. To keep the pH value at favorite range, pH arrangement should be applied.

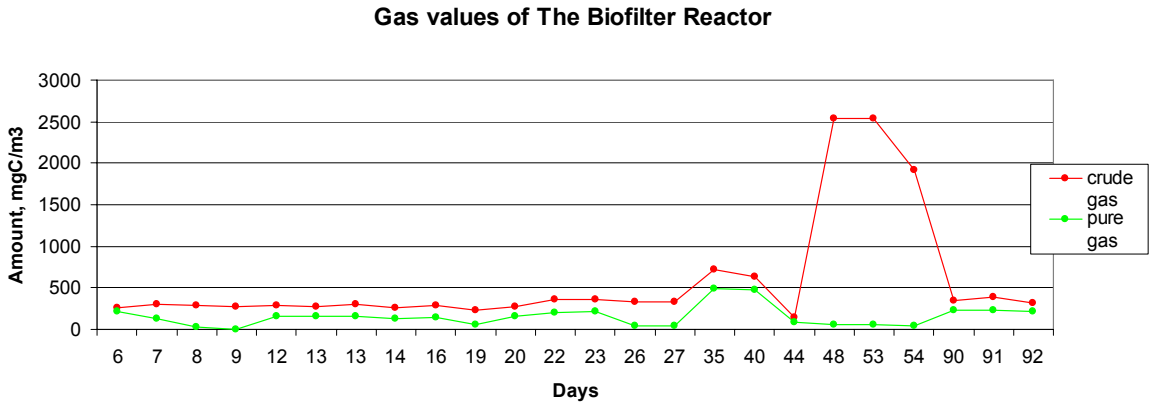

Figure 4.16: Daily Crude and Pure gas values of Bioreactor

It can be said that Bioreactor works in somehow stable efficiency when caring the pH changes. At the beginning to inoculation after adaptation period it works better. Its efficiency fluctuates with condition of microorganisms (i.e. when pH value is low, microorganism corrosion occurs (Figure 4.17 and 4.18). BAL accumulation also observed in Bioreactor eluent)

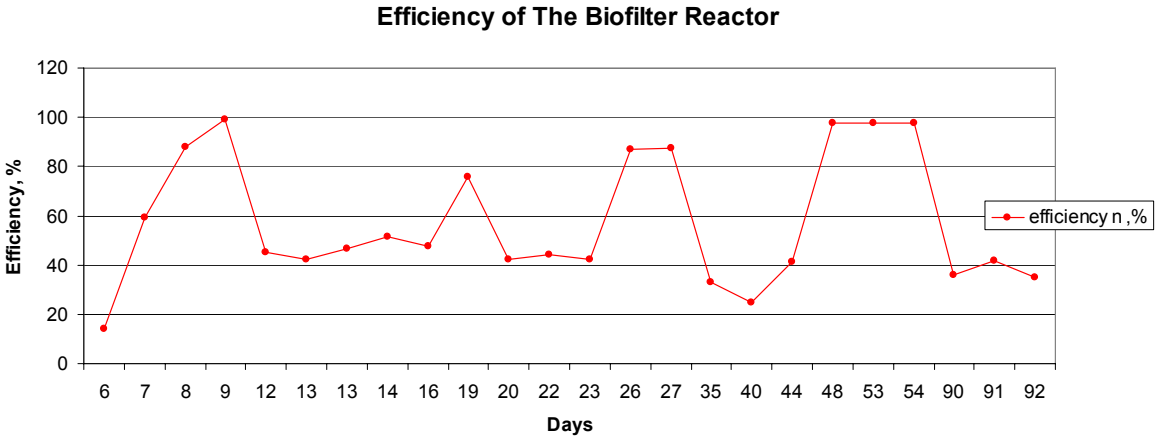

Figure 4.17: Daily efficiency values of Bioreactor.

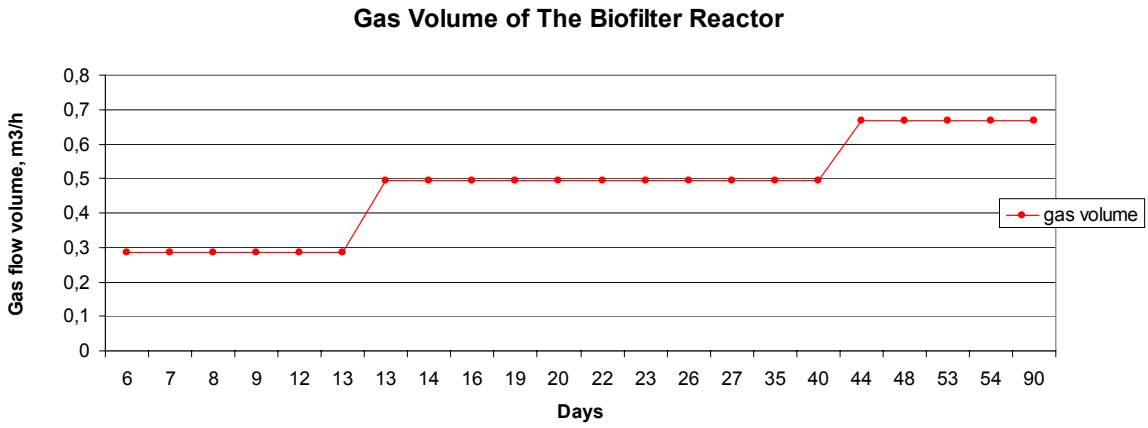

Figure 4.18: Daily gas volume values of Bioreactor.

Gas volume increased to values of 0,285, 0.493, 0.667 m^3/h to compensate desired high load.

In graph, interpreting specific degradation rate line it is concluded that reactor shows coherence to the load (Figuer 4.19).

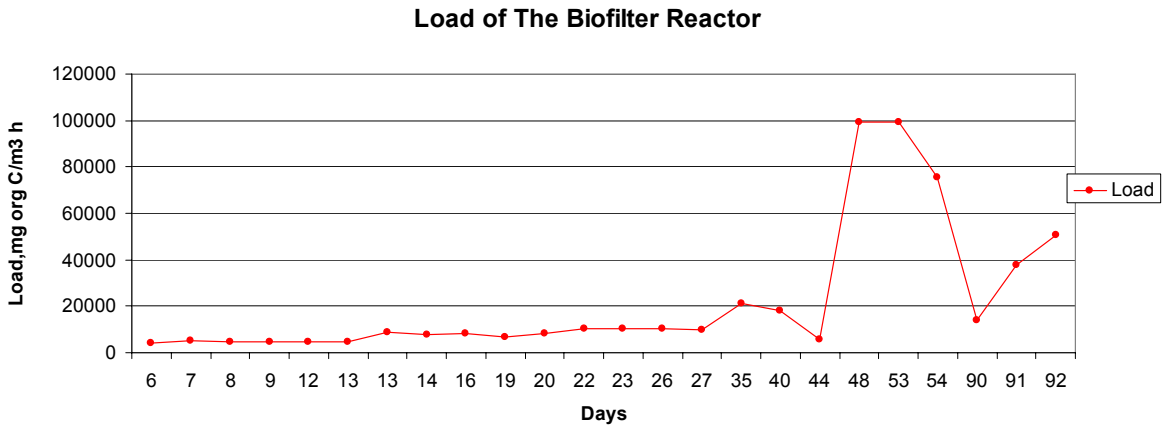

Figure 4.19: Daily substrate load values of Bioreactor.

In graph, interpreting specific degradation rate line it is concluded that reactor shows coherence to the load (Figure 4.20).

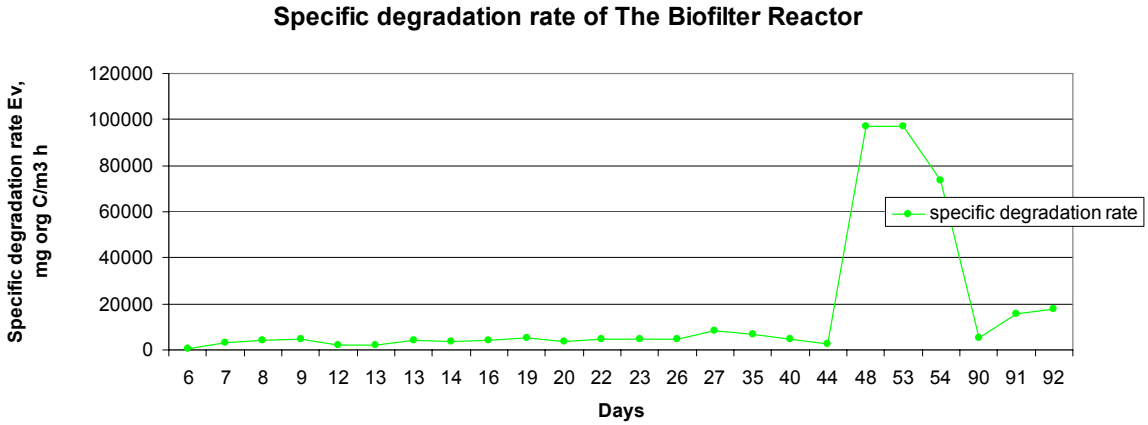

Figure 4.20: Daily specific degradation rate values of Bioreactor.

Effect of benzyl alcohol onto the material used for the bioreactor is shown in Figure 4.21.

Figure 4.21: Effects of Benzyl alcohol onto the materials.

5. CONCLUSION AND RECOMENDATION

Simulating and monitoring of the system is main duty of this study. To achieve this aim, isolated species were inoculated into the system. Moreover, their behaviours in the system were observed (i.e. pH change, temperature effect, efficiency, tolerance to gas flow amount, load, specific degradation rate and substrate amount in the eluent). Isolation and Enrichment of Benzyl alcohol, Benzylaldehyde and Sodium benzoate Degrading Bacteria Species and observing the performance of reactor took much time in this study and also were more difficult part of this work.

During the simulation of lab scale biofilter, to achieve desired load concentration of aromatic carbon different combination of heaters and pumps are used. Due to the elevated boiling point of Benzyl alcohol to get saturated air flow was main constrain in the study.

The origin of the sample is crucial for the success of enrichment of aromatic hydrocarbon degrading bacteria. In this work, samples from which are upper soil layer are collected around gas station were used as an inocculum.
In this first step of enrichment, a lot of colonies of benzyl alcohol and benzaldehyde degrading bacteria were yielded. The species were examined by classical microbiological tests.

According to determination of microorganism number on the used agar plate, the number of Benzylaldehyde species is about 10 times more than that of Benzyl alcohol species. These numbers are important due to indication of their degradation abilities.

Different benzyl alcohol and benzylaldehyde species are compared to their growth rate and degradation abilities (i.e. metabolism rate) using the benzyl alcohol as a substrate. By this way, the four fastest growing species were selected, studied further and inoculated into the bioreactor.

The Benzyl alcohol 200 species is an aerobic gram negative rod shaped bacteria. The Benzyl alcohol 215 species is an aerobic gram negative rod shaped bacteria. The

Benzylaldehyde 900 and 930 species are an aerobic gram negative spherical bacteria. This description of the species is important in this study regarding the aim of degradation of aromatic hydrocarbons. For this aim gram negative Identification test (BIOLOG) and API 20 NE test were performed. Both tests base on degradation capabilities. The results are given in result part of this study. More important is that they are not same at description of microorganism. Especially, API 20 NE test describes absolutely all species as *Pseudomonas cepacia*. The tests use the same principle, but they use different chemicals and database. According to the literature survey and the descriptions of micro organisms which had already done, when performing experiments with them, species should be handled with care.

Benzyl alcohol and benzylaldehyde species are growing at a rate between 1,7 h^{-1} and 3,4 h^{-1} or consuming benzyl alcohol at a rate of 378 mg per liter, hour and optical density and consuming Benzylaldehyde at a rate 95 mg/(LhOD). Therefore they are good choice to used them in biofilter system. Although Benzylaldehyde degradation is the third step of degradation of aromatic hydrocarbon starting toluene, its degradation rate is less than that of Benzyl alcohol.

Regarding to HPLC analysis, growth medium showed that degradation of Benzylalcohol by benzylalcohol 200 species is total at a given concentration of 2 mmol/L in 3,5 h and degradation of Benzylaldehyde by Benzylaldehyde 900 and 930 species are total at a given concentration of 2 mmo/L in 1 and 1,5 h, so almost no intermediate metabolite appear in the eluent or on the filter material after the growth.

Possible hazard of this species to human is ranging little medical risk to the dangerous pathogens.

Acinetobacter calcoaceticus/genospecies 3 observed in generally in the hospital environment It causes hospital-acquired respiratory, urinary tract, wound infections, abscesses, and meningitis in debilitated humans.

A. baumanni/genospecies 2 is an opportunistic pathogen operating in hospitals creating serious infections such as pneumonia.

B. cepacia poses little medical risk to healthy people.

The most important experience of this study is that Benzylalcohol is really aggressive in different materials. This causes serious problem in caring bioreactor and its surroundings.

One should care construction materials when builing industrial scale biofilter reactor. It destroys metals, glass and even epoxy flour coatings.

The other important experience of this study is that benzylalcohol and benzylaldehyde degrading bacteria need really gentle lag phase to adapt the stream of Benzyl alcohol. Growth phase need more time. No care of this phase leads accumulation of Benzyl alcohol and decrease in growth of biology to contradiction of growth experiments. Possible reason for this is toxicity of Benzyl alcohol to species. It is recommended that loading should be started with low concentration of benzyl alcohol and increased gently to have good growth ability. The reactor should have large number of cell inoculated to have good biozoenosis.

It is observed in the eluent of biofilter that increasing concentration of Benzyl alcohol also caused increase of the benzylaldehyde, acetate and benzoate concentration. This also shows that the degradation of Benzyl alcohol follows the well-known as it documented in http://umbbd.ahc.umn.edu degradation pathway of Toluene. But the increase in benzoate concentration in the eluent causes an significant increase of solution`s pH. This effect is observed at the concentration of 1,2 g Benzoate/L. This create dangerous situation to biofliter materials. These are degradation of biofilter materials and losing microorganism activity due to death of them. Observed results indicate that main problem in the system is sudden acidification to pH 4.5. This leads to the death of the bacteria. To keep the pH value at favorite range, pH arrangement should be applied.

Following problem is accumulation of benzylalcohol up to the its saturation limit in water and naturally corrosion of the biofilter. It also caused the failure of running pump. The pump should be having chemical resistance.

Beyond this point the smell is another problem.
Bioreactor efficiency fluctuates with condition of microorganisms (i.e. when pH value is low, microorganism corrosion occurs. BAL accumulation also observed in Bioreactor eluent).

There is sometimes no strictly correlation between pH value and efficiency result when the graphics which are obtained are interpreted. This may be caused by a black box effect. There is need to construct more well designed set ups or repeating the whole experiments again. Efficiency values are generally fluctuates.

Working with a biofilter reactor is sometimes difficult as in case of Benzyl alcohol degradation. One will also need pH regulating device and continuous pH monitoring.

Only looking and comparing the values of crude gas concentration and pure gas concentrations during the collection of data is not valid (Table 7). An increase in crude gas concentration also causes an increase in pure gas concentration. But load and specific degradation rate graphics give easily needed conclusions. That is why one should get load and specific degradation rate graphics.

In some degradation tests substrate amount (i.e.2,2 mmol/L) is higher than the added amount(i.e.2 mmol/L). Degradation test of Benzylalcohol 200 species is an example for this situation. The reason for that should be preparing sample and measuring device (i.e. HPLC) failures.

To carry the species to the site of industrial bioreactor, their ability to growth in Sodium benzoate is measured. They have this ability.

Regarding to recommendations for a successful of waste air cleaning of benzyl alcohol:

Using biotrickling filter instead of biofilter..

Achieve to keep filter material in moisture medium.

Starting phase of inoculation can be critical.

Fertilization with phosphor and nitrogen is obligatory, since the drainage water for constructional reasons cannot be led in the circle.

To keep the pH value at favorite range, pH arrangement should be applied.

The operating condition of the plant must be supervised.

REFERENCES

A. Basu, S. S. Dixit, and P. S. Phale., 2003. Metabolism of benzyl alcohol via catechol *ortho*-pathway in methylnaphthalene-degrading *Pseudomonas putida* CSV86. *Appl. Microbiol. Biotechnol.*, **62,** 579-585.

A. D. Wouter, S. Marques, B. Wind, J. L. Ramos, and J. G. van Andel.,1996. Catabolite repression of the toluene degradation pathway in *Pseudomonas putida* harboring pWW0 under various conditions of nutrient limitation in chemostat culture. *Appl. Envir. Microbiol*., **62,** 601-606.

AE Mars, J Houwing, J Dolfing, and DB Janssen., Mar 1996. Degradation of Toluene and Trichloroethylene by Burkholderia cepacia G4 in Growth-Limited Fed-Batch Culture. *Appl. Envir. Microbiol.* **62,** 886 - 891.

A Massol-Deya, R Weller, L Rios-Hernandez, JZ Zhou, RF Hickey, and JM Tiedje., Jan 1997. Succession and convergence of biofilm communities in fixed-film reactors treating aromatic hydrocarbons in groundwater. *Appl. Envir. Microbiol.,* **63,** 270 – 276.

Alison Buchan, Lauren S. Collier, Ellen L. Neidle, and Mary Ann Moran., Nov 2000. Key Aromatic-Ring-Cleaving Enzyme, Protocatechuate 3,4-Dioxygenase, in the Ecologically Important Marine *Roseobacter* Lineage. *Appl. Envir. Microbiol.,* **66,** 4662 - 4672.

Bjarke B. Christensen, Janus A. J. Haagensen, Arne Heydorn, and Søren Molin., May 2002. Metabolic Commensalism and Competition in a Two-Species Microbial Consortium. *Appl. Envir. Microbiol.,* **68,** 2495 - 2502.

Blanaid Mee, Dermot Kelleher, Jesus Frias, Renee Malone, Keith F. Tipton, Gary T.M. Henehan, and Henry J. Windle., Mar 2005. Characterization of cinnamyl alcohol dehydrogenase of *Helicobacter pylori*: An aldehyde dismutating enzyme. *FEBS J.,* **272,** 1255 - 1264.

Bruno Bühler, Andreas Schmid, Bernhard Hauer, and Bernard Witholt., Mar 2000. Xylene Monooxygenase Catalyzes the Multistep Oxygenation of Toluene and Pseudocumene to Corresponding Alcohols, Aldehydes, and Acids in *Escherichia coli* JM101. *J. Biol. Chem.,* **275,** 10085 - 10092.

Carmen Lapadatescu, Christian Giniès, Jean-Luc Le Quéré, and Pascal Bonnarme., Apr 2000. Novel Scheme for Biosynthesis of Aryl Metabolites from L-Phenylalanine in the Fungus *Bjerkandera adusta. Appl. Envir. Microbiol.,* **66,** 1517 - 1522.

Charles E. Cowles, Nancy N. Nichols, and Caroline S. Harwood., Nov 2000. BenR, a XylS Homologue, Regulates Three Different Pathways of Aromatic Acid Degradation in *Pseudomonas putida. J. Bacteriol.,* **182,** 6339 - 6346.

Cox, H.H.J., and M.A. Deshusses. 2002. Biotrickling Filters for Air Pollution Control. p. 782-795. Vol. 2. In G. Bitton (Editor-in-Chief), The Encyclopedia of Environmental Microbiology. J. Wiley & Sons

Dale A. Pelletier and Caroline S. Harwood., May 2000. 2-Hydroxycyclohexanecarboxyl Coenzyme A Dehydrogenase, an Enzyme Characteristic of the Anaerobic Benzoate Degradation Pathway Used by *Rhodopseudomonas palustris. J. Bacteriol.,* **182,** 2753 - 2760.

Didier Moissenet, Philippe Bidet, Antoine Garbarg-Chenon, Guillaume Arlet, and Hoang Vu-Thien., Jan 2001. *Ralstonia paucula* (Formerly CDC Group IV c-2): Unsuccessful Strain Differentiation with PCR-Based Methods, Study of the 16S-23S Spacer of the rRNA Operon, and Comparison with Other *Ralstonia* Species (*R. eutropha*, *R. pickettii*, *R. gilardii*, and *R. solanacearum*). *J. Clin. Microbiol.,* **39,** 381 - 384.

E. A. Edwards, L. E. Wills, M. Reinhard, and Grbic-Galic., Mar 1992. Anaerobic degradation of toluene and xylene by aquifer microorganisms under sulfate-reducing conditions. *Appl. Envir. Microbiol.,* **58,** 794 – 800.

Enrico Baciocchi, Maura Fabbrini, Osvaldo Lanzalunga, Laura Manduchi, and Giorgio Pochetti., Feb 2001. Prochiral selectivity in H_2O_2-promoted oxidation of arylalkanols catalysed by chloroperoxidase: The role of the interactions between the OH group and the amino-acid residues in the enzyme active site. *Eur. J. Biochem.,* **268,** 665 - 672.

Ersilia Griva, Enrica Pessione, Sara Divari, Francesca Valetti, Maria Cavaletto, Gian Luigi Rossi, and Carlo Giunta., Apr 2003. Phenol hydroxylase from *Acinetobacter radioresistens* S13: Isolation and characterization of the regulatory component. *Eur. J. Biochem.,* **270,** 1434 - 1440.

Faith H. Harrison and Caroline S. Harwood., Mar 2005. The *pimFABCDE* operon from *Rhodopseudomonas palustris* mediates dicarboxylic acid degradation and participates in anaerobic benzoate degradation. *Microbiology,* **151,** 727 - 736.

Fernando J. Irazoqui, Pablo H. H. Lopez, Miguel A. Vides, and Gustavo A. Nores., Aug 2000. Novel immunogenicity of Thomsen-Friedenreich disaccharide obtained by a molecular rotation on its carrier linkage. *Glycobiology,* **10,** 781 - 787.

Fumika Kamada, Suzuna Abe, Nobuhiro Hiratsuka, Hiroyuki Wariishi, and Hiroo Tanaka., Jun 2002. Mineralization of aromatic compounds by brown-rot basidiomycetes – mechanisms involved in initial attack on the aromatic ring. *Microbiology,* **148,** 1939 - 1946.

Hikaru Suenaga, Mariko Mitsuoka, Yuko Ura, Takahito Watanabe, and Kensuke Furukawa., Sep 2001. Directed Evolution of Biphenyl Dioxygenase: Emergence of Enhanced Degradation Capacity for Benzene, Toluene, and Alkylbenzenes. *J. Bacteriol.,* **183,** 5441 – 5444.

Horacio Bach, Yevgeny Berdichevsky, and David Gutnick., May 2003. An Exocellular Protein from the Oil-Degrading Microbe *Acinetobacter venetianus* RAG-1 Enhances the Emulsifying Activity of the Polymeric Bioemulsifier Emulsan. *Appl. Envir. Microbiol.,* **69,** 2608 - 2615.

Inés García-Peña, Sergio Hernández, Richard Auria, and Sergio Revah., August 2005. Correlation of Biological Activity and Reactor Performance in Biofiltration of Toluene with the Fungus *Paecilomyces variotii* CBS115145. *Appl. Envir. Microbiol.,* **71,** 4280 - 4285.

Inez J. T. Dinkla, Esther M. Gabor, and Dick B. Janssen., Aug 2001. Effects of Iron Limitation on the Degradation of Toluene by *Pseudomonas* Strains Carrying the TOL (pWWO) Plasmid. *Appl. Envir. Microbiol.,* **67,** 3406 - 3412.

Jennifer Boatright, Florence Negre, Xinlu Chen, Christine M. Kish, Barbara Wood, Greg Peel, Irina Orlova, David Gang, David Rhodes, and Natalia Dudareva., Aug 2004. Understanding in Vivo Benzenoid Metabolism in Petunia Petal Tissue. *Plant Physiology,* **135,** 1993 - 2011.

John C. D'Auria, Feng Chen, and Eran Pichersky., Sep 2002. Characterization of an Acyltransferase Capable of Synthesizing Benzylbenzoate and Other Volatile Esters in Flowers and Damaged Leaves of *Clarkia breweri. Plant Physiology,* **130,** 466 - 476.

Katrin Pollmann, Stefan Beil, and Dietmar H. Pieper., Sep 2001. Transformation of Chlorinated Benzenes and Toluenes by *Ralstonia* sp. Strain PS12 *tecA* (Tetrachlorobenzene Dioxygenase) and *tecB* (Chlorobenzene Dihydrodiol Dehydrogenase) Gene Products. *Appl. Envir. Microbiol.,* **67,** 4057 - 4063.

Keith D. James, Michelle A. Hughes, and Peter A. Williams., Jun 2000. Cloning and Expression of *ntnD*, Encoding a Novel $NAD(P)^+$-Independent 4-Nitrobenzyl Alcohol Dehydrogenase from *Pseudomonas* sp. Strain TW3. *J. Bacteriol.,* **182,** 3136 - 3141.

Kelly D. Goodwin, Ryszard Tokarczyk, F. Carol Stephens, and Eric S. Saltzman., July 2005. Description of Toluene Inhibition of Methyl Bromide Biodegradation in Seawater and Isolation of a Marine Toluene Oxidizer That Degrades Methyl Bromide. *Appl. Envir. Microbiol.,* **71,** 3495 - 3503.

Magdy El-Said Mohamed, Annette Zaar, Christa Ebenau-Jehle, and Georg Fuchs., Mar 2001. Reinvestigation of a New Type of Aerobic Benzoate Metabolism in the Proteobacterium *Azoarcus evansii. J. Bacteriol.,* **183,** 1899 - 1908.

Maria-Angeles Abril, Carmen Michan, Kenneth N. Timmis, and Juan L. Ramos., Dec 1989. Regulator and enzyme specificities of the TOL plasmid-encoded upper pathway for degradation of aromatic hydrocarbons and expansion of the substrate range of the pathway. *J. Bacteriol.,* **171,** 6782 – 6790.

María J. López Barragán, Manuel Carmona, María T. Zamarro, Bärbel Thiele, Matthias Boll, Georg Fuchs, José L. García, and Eduardo Díaz., Sep 2004. The *bzd* Gene Cluster, Coding for Anaerobic Benzoate Catabolism, in *Azoarcus* sp. Strain CIB. *J. Bacteriol.,* **186,** 5762 - 5774.

Markus Göbel, Kerstin Kassel-Cati, Eberhard Schmidt, and Walter Reineke., Jan 2002. Degradation of Aromatics and Chloroaromatics by *Pseudomonas* sp. Strain B13: Cloning, Characterization, and Analysis of Sequences Encoding 3-Oxoadipate:Succinyl-Coenzyme A (CoA) Transferase and 3-Oxoadipyl-CoA Thiolase. *J. Bacteriol.,* **184,** 216 - 223.

Michael F. Wilkemeyer, Carrie E. Menkari, and Michael E. Charness., Nov 2002. Novel Antagonists of Alcohol Inhibition of L1-Mediated Cell Adhesion: Multiple Mechanisms of Action. *Mol. Pharmacol.,* **62,** 1053 - 1060.

MS Shields, MJ Reagin, RR Gerger, R Campbell, and C Somerville., Apr 1995. TOM, a new aromatic degradative plasmid from Burkholderia (Pseudomonas) cepacia G4. *Appl. Envir. Microbiol.,* **61,** 1352 - 1356.

Peter Schenkels, and Johannis A. Duine., Apr 2000. Nicotinoprotein (NADH-containing) alcohol dehydrogenase from *Rhodococcus erythropolis* DSM 1069: an efficient catalyst for coenzyme-independent oxidation of a broad spectrum of alcohols and the interconversion of alcohols and aldehydes. *Microbiology,* **146,** 775 - 785.

Rebecca E. Parales, Jayna L. Ditty, and Caroline S. Harwood., Sep 2000. Toluene-Degrading Bacteria Are Chemotactic towards the Environmental Pollutants Benzene, Toluene, and Trichloroethylene. *Appl. Envir. Microbiol.,* **66,** 4098 - 4104.

René Ullrich, Jörg Nüske, Katrin Scheibner, Jörg Spantzel, and Martin Hofrichter., Aug 2004. Novel Haloperoxidase from the Agaric Basidiomycete *Agrocybe aegerita* Oxidizes Aryl Alcohols and Aldehydes. *Appl. Envir. Microbiol.,* **70,** 4575 - 4581.

Rheinallt M. Jones and Peter A. Williams., Jan 2001. *Are CBA* Is an Operon in *Acinetobacter* sp. Strain ADP1 and Is Controlled by AreR, a σ^{54}-Dependent Regulator. *J. Bacteriol.,* **183,** 405 - 409.

Rheinallt M. Jones and Peter A. Williams., Sep 2003. Mutational Analysis of the Critical Bases Involved in Activation of the AreR-Regulated σ^{54}-Dependent Promoter in *Acinetobacter* sp. Strain ADP1. *Appl. Envir. Microbiol.,* **69,** 5627 - 5635.

Robert M. Zucker, Aparna P. Keshaviah, Owen T. Price, and Jerome M. Goldman., Jun 2000. Confocal Laser Scanning Microscopy of Rat Follicle Development. *J. Histochem. Cytochem.,* **48,** 781 - 792.

Robert Witzig, Howard Junca, Hans-Jürgen Hecht, and Dietmar H. Pieper, 2006. Assessment of Toluene/Biphenyl Dioxygenase Gene Diversity in Benzene-Polluted Soils: Links between Benzene Biodegradation and Genes Similar to Those Encoding Isopropylbenzene Dioxygenases. *Appl. Envir. Microbiol.* **72,** 3504-3514.

Romy Chakraborty and John D. Coates., September 2005. Hydroxylation and Carboxylation—Two Crucial Steps of Anaerobic Benzene Degradation by *Dechloromonas* Strain RCB. *Appl. Envir. Microbiol.,* **71,** 5427 - 5432.

Salvador Genovés, José V. Gil, Salvador Vallés, José A. Casas, and Paloma Manzanares. Jun 2005. Assessment of the Aromatic Potential of Palomino Fino Grape Must Using Glycosidases. *Am. J. Enol. Vitic.,* **56,** 188 - 191.

Shiue-Shien Weng, Mei-Wen Shen, Jun-Qi Kao, Yogesh S. Munot, and Chien-Tien Chen., Mar 2006. Chiral *N*-salicylidene vanadyl carboxylate-catalyzed enantioselective aerobic oxidation of α-hydroxy esters and amides. *PNAS,* **103,** 3522 - 3527.

Simon Kühner, Lars Wöhlbrand, Ingo Fritz, Wasco Wruck, Claus Hultschig, Peter Hufnagel, Michael Kube, Richard Reinhardt, and Ralf Rabus., Feb 2005. Substrate-Dependent Regulation of Anaerobic Degradation Pathways for Toluene and Ethylbenzene in a Denitrifying Bacterium, Strain EbN1. *J. Bacteriol.,* **187,** 1493 - 1503.

Stefan Uthoff, Tim Stöveken, Nikolaus Weber, Klaus Vosmann, Erika Klein, Rainer Kalscheuer, and Alexander Steinbüchel., Feb 2005. Thio Wax Ester Biosynthesis Utilizing the Unspecific Bifunctional Wax Ester Synthase/Acyl Coenzyme A:Diacylglycerol Acyltransferase of *Acinetobacter* sp. Strain ADP1. *Appl. Envir. Microbiol.,* **71,** 790 - 796.

V. J. Denef, J. Park, T. V. Tsoi, J.-M. Rouillard, H. Zhang, J. A. Wibbenmeyer, W. Verstraete, E. Gulari, S. A. Hashsham, and J. M. Tiedje., Aug 2004. Biphenyl and Benzoate Metabolism in a Genomic Context: Outlining Genome-Wide Metabolic Networks in *Burkholderia xenovorans* LB400. *Appl. Envir. Microbiol.,* **70,** 4961 - 4970.

V. J. Denef, J. A. Klappenbach, M. A. Patrauchan, C. Florizone, J. L. M. Rodrigues, T. V. Tsoi, W. Verstraete, L. D. Eltis, and J. M. Tiedje., January 2006. Genetic and Genomic Insights into the Role of Benzoate-Catabolic Pathway Redundancy in *Burkholderia xenovorans* LB400. *Appl. Envir. Microbiol.,* **72,** 585 - 595.

Wataru Kitagawa, Keisuke Miyauchi, Eiji Masai, and Masao Fukuda., Nov 2001. Cloning and Characterization of Benzoate Catabolic Genes in the Gram-Positive Polychlorinated Biphenyl Degrader *Rhodococcus* sp. Strain RHA1. *J. Bacteriol.,* **183,** 6598 - 6606.

Xuan Zhong Li, Jeremy S. Webb, Staffan Kjelleberg, and Bettina Rosche., February 2006. Enhanced Benzaldehyde Tolerance in *Zymomonas mobilis* Biofilms and the Potential of Biofilm Applications in Fine-Chemical Production. *Appl. Envir. Microbiol.,* **72,** 1639 - 1644.

Ying Tao, Ayelet Fishman, William E. Bentley, and Thomas K. Wood., Jul 2004. Oxidation of Benzene to Phenol, Catechol, and 1,2,3-Trihydroxybenzene by Toluene 4-Monooxygenase of *Pseudomonas mendocina* KR1 and Toluene 3-Monooxygenase of *Ralstonia pickettii* PKO1. *Appl. Envir. Microbiol.,* **70,** 3814 - 3820.

Yong Ge and Lindsay D. Eltis., Sep 2003. Characterization of Hybrid Toluate and Benzoate Dioxygenases. *J. Bacteriol.,* **185,** 5333 - 5341.

www.uk.chemdat.info/mda/uk

http://europa.eu/scadplus/leg/en/lvb/l28029b.htm

CURRICULUM VITAE

Oktay Karaş was born in Trabzon in 1969. He graduated from Şehremini High School in 1978. He received his B.Sc.degree in Chemistry from Middle East Technical University in 1994. He started studying for his master's degree in 2003 in Environmental Biotechnology Programme of İstanbul Technical University.