ISTANBUL TECHNICAL UNIVERSITY ★ GRADUATE SCHOOL OF SCIENCE ENGINEERING AND TECHNOLOGY

REMOVAL OF ODOR EMISSIONS FROM FOOD FERMENTATION AND PETROCHEMICAL PRODUCTION PROCESSES WITH USING BIOLOGICAL TREATMENT METHODS

PhD. THESIS

İlker AKMIRZA

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İlker AKMIRZA
(501122705)

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Thesis Advisor: Prof. Dr. Kadir ALP
Thesis Co-Advisor: Prof. Dr. Raul MUNOZ TORRE

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İSTANBUL TEKNİK ÜNİVERSİTESİ ★ FEN BİLİMLERİ ENSTİTÜSÜ

GIDA FERMENTASYON VE PETROKİMYA ÜRETİM SEKTÖRLERİ KAYNAKLI KOKU EMİSYONLARININ BİYOLOJİK ARITMA YÖNTEMLERİ İLE GİDERİLMESİ

DOKTORA TEZİ

İlker AKMIRZA
(501122705)

Çevre Mühendisliği Anabilim Dalı

Çevre Bilimleri, Mühendisliği ve Yönetimi Programı

Tez Danışmanı: Prof. Dr. Kadir ALP
Eş Danışman: Prof.Dr. Raul Munoz TORRE

ŞUBAT 2019

İlker Akmırza, a Ph.D. student of İTU Graduate School of Science Engineering and Technology student ID 501122705, successfully defended the thesis entitled “Removal Of Odor Emissions From Food Fermentation And Petrochemical Production Processes With Using Biological Treatment Methods”, which he prepared after fulfilling the requirements specified in the associated legislations, before the jury whose signatures are below.

Thesis Advisor : **Prof. Dr. Kadir ALP** ....
Istanbul Technical University

Co-advisor : **Prof.Dr. Raul Munoz TORRE** ....
Valladolid University

Jury Members : **Prof. Dr. İsmail TORÖZ** ....
Istanbul Technical University

Prof. Dr. İsmail KOYUNCU ....
İstanbul Technical University

Prof. Dr. Hayrettin Güçlü İNSEL ....
Istanbul Technical University

Assoc. Prof. Dr. Mustafa TÜRKER ....
PAKMAYA

Assist. Prof. Dr. Raquel LEBRERO ....
Valladolid University

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To my family,

FOREWORD

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ABBREVIATIONS

CERCLA **:** The Comprehensive Environmental Response, Compensation, and Liability Act

EPA **:** Environmental Protection Agency

SYMBOLS

BTEX : Benzene-toluene-ethylbenzene-xylene
C_o : Initial pollutant concentration (mg m^{-3})
C_i : Pollutant concentration at time (mg m^{-3})
D : Cumulative degradation (-)
D_{max} : Maximum degradation potential (mg mg^{-1})
DGGE : Denaturing gradient gel electrophoresis **(-)**
GC-TCD : Gas Chromatography with Thermal Conductivity Detector
GC-FID : Gas Chromatography with Flame Ionization Detector
H : Shannon-Wiener diversity index (-)
K_I : Inhibition parameter (mg m^{-3})
K_S : Half-velocity parameter (mg m^{-3})
MSM : Mineral salt medium (-)
P : Cumulative production (mg m^{-3})
P_{max} : Maximum cumulative production (mg m^{-3})
PCR : Polymerase chain reaction (-)
R_m : Maximum rate of production (mg mg^{-1} s^{-1})
S : Concentration of the limiting growth substrate (mg m^{-3})
t_∞ : End time (negligible variation of pollutantconcentration)(s)
VOC : Volatile organic compounds (-)
VSS : Volatile suspended solids (kg m^{-3})
X : Total biomass concentration (mg m^{-3})
Y_{xs} : Biomass yield coefficient (mg mg^{-1})
μ_{max} : Maximum specific growth rate (s^{-1})

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REMOVAL OF ODOR EMISSIONS FROM FOOD FERMENTATION AND PETROCHEMICAL PRODUCTION PROCESSES WITH USING BIOLOGICAL TREATMENT METHODS

SUMMARY

Air pollution is one such form that refers to the contamination of the air, irrespective of indoors or outside but until now it tooks less attention than other forms of pollution such as water or soil pollution. However, the world struggled with environmental challenges and problems related to air pollution. In recent days air pollution is considered a major environmental problem and it comes in a variety of forms, from visible particles of soot or smoke to invisible gases such as sulfur dioxide and carbon monoxide. Although some sources of atmospheric pollution are emitted naturally, from volcanoes or forest fires, most are anthrophogenic sources and originated from human activity in the home or workplace.

Among the air pollutants nowadays odor emissions are considered as an important air pollution parameters. Although odor and volatile organic compound (VOC) emissions is tightly depended on human sensation and also in some cases can not be detected at low concentration by society, many inorganic and organic chemical compounds take part in odor structure and odor emissions include toxic materials on the human and environmental health even at trace concentrations.

Important part of VOC which take part in odor formation trigger ozone synthesis in the atmosphere and contribute to the greenhouse gases with the oxidation products. Apart the health and environmental effect of odorous air emissions they have also irritant effect on human sense and cause odor nuisanse in surrounding residental areas of odor source and long term exposure to these emissions result on people to symptoms prevalence of emotional stresses. The wide range of release these VOCs and malodorus from industries waste management, livestock facilities or agriculture makes the solution of the problem prominent.

Especially industrial revolution resulted in increasingly larger scale manufacturing and industrial operations that significantly increased the air pollution levels. These operations required increasingly larger sources of energy, usually from the burning of fossil fuels. They released gases, vapors, and particulates to the atmosphere.

For proper and effective odor abatement for selected industry branches to be implemented, first of all the problem must be quantified, and source characteristic should be defined. However, in literature there is a lack of comparative data assessing the proper characterization of VOCs and malodous compounds. In consequence of sensorial impact of malodous VOC emissions in many cases instrumental techniques such as gas chromatogram coupled mass spectrometry doesnt enough to understand proper characterization escpecially direct impact on people. Within the use of sensorial technique of dynamic olfactometry human nose used as a sensor and allows to determine the threshold of odor and concentration/intensity of odor emissions and allow to determine direct impact of malodous VOCs on people. Within the use of

instrumental and olfactometric techniques simultaneously, waste gas characterization can be determine in both objective and subjective points of view and selection of proper odor abatement techniques for characterized waste gas emissions can be put one’s finger.

Apart the industries yeast fermentation process has been used in wide range for making bread beer since ancient ages and releases malodorous emissions from its fermentation proses to atmosphere and results odor nuisance in sorrounding residental areas. Despite the specific features of malodorous VOC emissions in the proses, all of them are susceptible to be treated by end-of-the pipe technologies sharing the same fundamental basis. Hence, even state-of-the-art physical/chemical technologies are either costly or inefficient when treating malodorous VOC emissions nowadays biological air pollution control technologies come into prominant due to their low operational and investment cost, environmental friendly nature and social acceptance but there is a lack of comparative data assessing for specified well-characterizad case studies

Within the first study of thesis work a case study was carried out for bakery yeast fermentation process and proses emissions were analyzed with both instrumental and olfactometric methods to illustrate malodorous VOC characterization in terms of both objective and subjective views and were biodegraded in a pilot scale biofilter. Instrumental analyses were performed to determine the chemical composition of emissions where ethanol was emitted as major VOC from the process and was followed by acetaldehyde and acetone and only trace propanol emissions were recorded only at peak hours of fermentation cycle. Simultaneusly to analytic analyses dynamic olfactometric analyses were performed to quantify the sensory impact of malodorous VOC emissions and the odor concentration was recorded 40 times higher concentration than announced legal limits.

At this point of concept biofilter selected as suitable alternative for the abatement of dilute VOC emissions less than 2000 mg m^{-3} and it has the waste gas treatment capacities for high flowrate over other technologies. Malodorus emissions were treated in a biofilter primarily for single ethanol load and achieved almost complete biodegradation while ternary waste gas stream simulation be provided within addition of acetaldehyde and acetone to synthetic stream and resulted higher than 90 % of ethanol, acetaldehyde removal where only partly acetone removal was observed. As a result of first study proper waste characterization adapted to case study for malodorus VOCs in both objective and subjective view and the superior performance of biofilter operation carried out for well-characterized bakery yeast fermentation process emissions for both single and ternary gas streams from process while still process improvement on acetone removal and VOC mass transfer enhancement for high waste gas flow will be required.

Petrochemical production processes is analysed as another model sector due to the association with the emission of various organic compounds to the atmosphere, mainly originating from the production processes, waste areas and the storage tanks. Up to date aerobic biological processes are frequently used to reduce the release odorous VOC emissions to ambient air, but they were strongly limited in O2-free petrochemical industry emissions due to their potential explosion risks and, cannot be implemented as end-of-pipe technologies. However, in recent days the number of studies devoted to O2-free BTEX removal is still scarce and little is known regarding the interactions among these pollutants during the degradation process. Therefore the kinetic parameter estimation come into prominant to evaluate the impacts of changes in

variables, such as operating conditions on system performances. In this regard, a part of the research carried out estimation kinetic parameters of BTEX under anoxic conditions to understand biodegradation pathways of microorganisms and help to overcome the problem of incorrect process design and modelling of biodegradation processes.

With the aim of the study typical waste water treatment plant activated sludge inoculum was being acclimated and used for the anoxic abatement of single, dual and quaternary BTEX mixtures. The results obtained demonstrated the differences during biodegradation of BTEX mixture while T and E were readily biodegradable on the contrast only partly biodegradation were achived for B and X. The mathematical models of Monod and a Modified Gompertz model were then used for the estimation of the biodegradation kinetic parameters including taking into account of substrate interactions for dual and quaternary mixtures, where Monod model provided an accurate description of the biodegradation of T and E, the Modified Gompertz model supported better fit for B and X degradation. Furthermore, the Gompertz model accurately represented the inhibitory interactions between BTEX mixtures, although it failed in representing the degradation of the recalcitrant compounds when all four BTEX were present simultaneously.

The last work is aimed to overcome the lack of use anoxic biodegradation of petroleum industry emissions as end pipe technology and to reduce potential explosion risks of aerobic conditions and reducing energy consumption for aeration. With the aim of this purpose an anoxic biotrickling filter operation carried out for the frequently petroleum industry emissions of BTEX and operation conditions were tested to improve the removal of BTEX mixture emissions via optimizing the operation parameters of pH, irrigation rate and adding UV as pretreatment to breakdown possible metabolites for selected emissions. Same biodegradation pathways were observed as kinetic parameter estimation of BTEX and T and E were found as readily biodegradable where only party biodegradation were observed for B and X for all operation conditions and considered as hardly biodegradable compounds under oxygen limited conditions. Also T was found as most sensetive compound to operation changes and substrate interactions resulted detrimental effect on anoxic biodegdaration. This research demonstrated for the first time biotreatment of BTEX under anoxic conditions and still need process improvement.

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ÖZET

Hava kirliliği, çevre ve iç ortam havasında bulunan kirleticilerin oluşturmuş olduğu kirliliğe verilen addır. Ancak hava kirliliği günümüze kadar su veya toprak kirliliği gibi diğer kirlilik türlerinden daha az dikkate alınan bir kirlilik türü olmuştur. Günümüzde ise dünya hava kirlenmesi kaynaklı bir çok çevresel sorunla karşı karşıya kalmaktadır ve hava kirliliği önemli bir çevresel sorun olarak kabul edilmektedir.

Hava kirliliği atmosferde gözle görülebilen partikül maddelerden gözle görülemeyen kükürt dioksit ve karbon monoksit gibi gazlara kadar geniş bir skaladaki kirleticilerin bulunması ile meydana gelmektedir. Her ne kadar hava kirliliği oluşturan volkan aktiviteleri, orman yaygınları gibi bazı doğal faaliyetler olmasına rağmen günümüzde kirlenme genellikle insan faaliyetleri sonucu ortaya çıkan kirlenmedir.

Hava kirlenmesine sebep olan emisyonlar içerisinde kokulu atık gaz emisyonları kayda değer bir önem taşımaktadır. Her ne kadar kokuya sebep olan uçucu madde bileşiklerin algılanma seviyesi insan algısına bağlı olmasına ve düşük konsantrasyonlarda zaman zaman tespit edilememesine rağmen, bir çok organik ve inorganik kimyasal madde kokulu atık gaz yapısında yer almakta ve de çevre ile insan sağlığına zararlı bir çok toksik kimyasalı iz konsantrasyonlarda dahi içermektedir.

Koku problemi oluşturan uçucu organik maddelerin önemli bir kısmı troposferde ozon sentezini tetiklemekte ve oksidasyon ürünleri ile sera gazı oluşumlarına sebebiyet vermektedir. Koku emisyonlarının sağlık ve çevresel etkilerinin yanı sıra bu emisyonların irrite edici özellikleri özellikle koku kaynaklarına yakın yerlerde yaşayan insanlarda koku şikayetlerinin artmasına ve çeşitli psikolojik sıkıntıların oluşmasına sebebiyet vermektedir.

Özellikle kokuya sebep olan emisyonların yaygın olarak atmosfere yayıldığı atık bertaraf tesisleri, tarımsal faaliyetler ve çeşitli endüstriyel faaliyetler koku probleminin çözümünü öncelikli kılmaktadır.

Günümüzde yaygınlaşan endüstriyelleşme ve buna paralel artış gösteren üretim ve de endüstriyel faaliyetler hava kirliliğinin artmasına önemli etkilerle katkıda bulunmuşlardır. Bu faaliyetler artan enerji ihtiyaçlarını genel olarak fosil yakıtlardan karşılarken buna paralel olarak çeşitli atıkgazları, buharları ve partiküler maddeleri atmosfere salmaktadır.

Tez çalışması kapsamında incelenen endüstri kollarında en uygun ve verimli koku emisyonu kontrol teknolojisinin seçiminin gerçekleştirilmesi, herşeyden önce koku probleminin tanımlanmasına ve koku kaynağının emisyon karakteristiğinin ortaya konmasına bağlı olmaktadır. Ancak bundan önce gerçekleştirilen çalışmalarda koku emisyonuna sebep olan uçucu organik maddelerinin karakterizasyonu ile ilgili noksanlıklar söz konusudur ve uygun karakterizasyon çalışmalarının gerçekleştirilmesi çok büyük önem taşımaktadır. Günümüzde koku emisyonlarının

sensörel etkisinden kaynaklı olarak yaygın olarak kullanılmakta olan gaz kromatografi kütle spektrometri metodu koku probleminin bireyler üzerindeki direk etkisini yansıtamamaktadır. Bu noktada kullanılmaya başlayan dinamik olfaktometri ölçüm metodu ile kokulu bileşiklerin bireyler üzerinde yaratmış olduğu etkiler ölçülebilinmektedir, ancak bu işlem kokulu gaz içeriği hakkında bir bilgi sunmamaktadır. Bu tez çalışması kapsamında simultene olarak gerçekleştirilen analitik ve olfaktometrik atıkgaz karakterizasyonu ile kokulu atıkgaz akımları hem objektif hem de subjektif parametreler cinsinden belirlenerek, kokulu atıkgaz akımları karakterize edilerek en uygun arıtma teknolojisinin seçilmesi sağlanmıştır.

Endüstri kolları içerisinde maya fermantasyon prosesi ekmek üretiminde yaygın olarak kullanılmakta ve fermentasyon prosesi sonucu bir çok kokulu bileşiği atmosfere salarak tesislere yakın yerleşim yerlerdinde koku problemlerinin oluşmasına sebebiyet vermektedir. Bir çok farklı karakterde kötü koku bileşik proses sonucu atmosfere yayılmakla beraber, bu bileşikler tek bir arıtma yöntemi ile arıtılmaya uygun bileşikleri içermektedir. Günümüze kadar yaygın olarak kullanılmakta olan fiziksel/kimyasal gaz arıtma teknolojileri yüksek arıtma maliyetleri ve düşük arıtma verimleri çerçevesinde yerlerini çevre dostu, düşük maliyetli, arıtma alternatiflerinden biyolojik arıtma yöntemlerine bırakmaktadır. Ancak farklı endüstri branşlarında oluşan atıkgazların arıtılmasına yönelik doğan farklılıklardan kaynaklı olarak uygulamlarda eksiklikler söz konusu olmaktadır.

Tez çalışmasının ilk bölümünde gerçekleştirilen saha çalışması ile ekmek mayası üretimi için gerçekleşen fermantasyon prosesi kokulu atıkgaz emisyonları enstrümental ve olfaktometrik analizler sonucunda objektif ve subjektif parametreler cinsinden karakterize edilmiştir. Karakterizasyon çalışması ile elde edilen atıkgaz akımlarının pilot ölçekli biyofiltre sisteminde arıtılması sağlanmıştır. Saha çalışması kapsamında enstrümental analizlar atıkgaz örneklern kimyasal içeriğini karakterize etme amacıyla gerçekleştirilmiş olunup, elde edilen analiz sonuçlarına göre proseste etanolün ana kokulu emisyon kaynağı olduğu belirlenirken, sistemde ayrıca asetaldehit ve aseton gibi bileşiklere rastlanmıştır ve de ayrıca eser miktarda propanol emisyonunun pik emisyon akımları içerisinde yer aldığı tespit edilmiştir. Analitik ölçümlerle aynı anda gerçekleştirilen dinamik olfaktometri analizleri ile de koku problemi oluşturan emisyonların bireyler üzerinde yaratmış olduğu etkiler belirlenmek istenmiş ve elde edilen sonuçlara göre koku emisyon konsantrasyonlarının yasal limitlerin yaklaşık 40 kat üstünde olduğu belirlenmiştir.

Bu bağlamda karakterize edilen proses atıkgaz akımı arıtımı için biyofiltre sisteminin proses akımında da ortaya çıkan 2000 mg m^{-3} den düşük konsantrasyonda ve yüksek atıkgaz debisindeki akımları arıtmak için diğer arıtma teknolojilerine göre daha uygun olduğu belirlenmiştir. Seçilen biyofiltre sistemi ilk önce kokulu atıkgaz akımlarında bulunan ana koku kaynağı bileşeni olan etanol için işletilmiş ve yaklaşık olarak tam verimle giderim sağlanırken; atık gaz içerisinde bulunan asetaldehit ve asetonunda atık gaz akımına eklenmesi sonucunda % 90 üzerinde etanol ve asetaldehit giderimi sağlanırken aseton ise kısmen sistemde biyodegradasyona uğramıştır. Tez çalışması kapsamında gerçekleştirelen bu ilk çalışma ile kokuya sebep olan uçucu organik maddelerin karakterizasyonu objektif ve subjektif parametreler cinsinden tespit edilmiş olunup, bu emisyonlar biyofiltre sisteminde yüksek verim ile tekil ve çoklu atıkgaz yüklemerinde biyolojik olarak arıtılmıştır. Ancak sistemde aseton arıtımı için özellikle yüksek atıkgaz debileri için kütle transferinin iyileştirilmesine ihtiyaç duyulmaktadır.

Tez çalışmasının gerçekleştiği bir diğer sektör olan petrokimya üretim sektörü özellikle üretim faaliyetleri, atık bertaraf alanları ve depolama tankları kaynaklı olarak bir çok kokulu atıkgaz akımını atmosfere salmaktadır. Günümüze kadar bir çok biyolojik arıtma sistemi aerobik koşullar altında kokulu atıkgaz akımlarının arıtılmasında yaygın olarak kullanılmıştır. Ancak bu noktada özellikle petrokimya endüstrisi emisyonlarının inert atmosferde bulunması ve oksijenin ortamda bulunduğu bölgelerde oluşan patlama riski, aerobik arıtma sistemlerinin kullanılmasını limitlemektedir.

Bu etklerin yanı sıra sıra sektörde yaygın olarak raslanan benzen, toluen, etilbenzen ve ksilen (BTEX) emisyonlarının çoklu emisyonlarının oksijensiz ortamlarda arıtılmasına ve arıtım esnasında bileşenlerin birbirleri üzerine olan etkilerine yönelik çok az sayıda çalışma mevcuttur.

Bu noktada tez çalışmasının ikinci kısmında BTEX emisyonlarının biyolojik arıtma faaliyetleri sırasında kinetik katsayılarının belirlenmesi amaçlanmış ve belirlenecek kinetik katsayılar çerçevesinde sistem arıtma performanslarınında kayda değer gelişmeler oluşturacağı belirlenmiştir. Gerçekleştirilen ikinci çalışma ile BTEX bileşenlerinin biyodegradasyon metabolik yolları belirlenerek uygun olmayan proses dizayn ve modellemesinin önüne geçilmek istenmiştir.

Çalışma kapsamında evsel atıksu arıtma tesisinde oluşan aşı çamuru anoksik koşullarda altında tekil, ikili ve dörtlü BTEX karışımları için aklime edilmiş ve bütün kombinasyonlar için kinetik katsayılarının belirlenmesi hedeflenmiştir. Elde edilen sonuçlar ışığında T ve E bileşiklerinin çok rahatlıkla biyolojik olarak ayrıştığı belirlenirken B ve X bileşiklerinin bunun tam tersine çok zor olarak biyolojik ayrıştığı tespit edilmiştir.Kinetik katsayıların belirlenmesi için matematik modeller olan Monod ve Modifiye Gompertz modelleri kullanılmış ve kinetik katsayılar belirlenirken çoklu kombinasyonlarda bileşenlerin arasındaki etkileşimin etkileri de dikkate alınmıştır. Çalışmalarda Monod matematik modelinin T ve E bileşenleri için çok büyük ölçüde uygunluk sağladığı belirlenirken, Modifiye Gompertz modeli ise biyolojik olarak zor ayrışan B ve X için daha temsil edici değerler sunmaktadır. Bunun yanı sıra Modifiye Gompertz modeli çoklu BTEX karışımları içerisindeki inhibisyon etkisini gösterebilirken (sadece dörtlü BTEX için düşük regrasyon değerleri), monod modeli bunu sağlayamamaktadır.

Tez çalışmasının üçüncü ve son kısmında ise tez çalışmasının ikinci çalışması kapsamında anoksik koşullar altında kinetik katsayıları belirlenen petrokimya üretim sektörlerinde yaygın olarak bulunan BTEX emisyonlarının oksijenin var olduğu koşullarda patlama riskinin önüne geçmek amacıyla, anoksik biyo damlatmalı filtre sisteminde arıtılması gerçekleştirilmştir. Böylelikle emisyonların yaygın olarak bulunduğu inert ortamda patlama riskinin önüne geçilmesinin yanı sıra havalandırma faaliyetleri için gerekli enerji ihtiyacının da azaltılması hedeflenmiştir.

Laboratuar ölçekli biyodamlatmalı filtre işletimi, sistem optimizasyonunun gerçekleştirilmesi için sistem pH, yıkama sıvısı hızı, UV eklemesi ile sistemde oluşabilecek potansiyel metabolitlerin ön arıtımı için uygulanması gibi bir çok farklı kararlı koşul altında işletilmiştir. Sistemde T ve E bileşenlerinin kinetik katsayıların belirlenmesinde olduğu gibi hızlıca biyolojik olarak ayrıştığı ve yüksek verimle arıtımın gerçekleştiği belirlenirken, B ve X‘in biyolojik olarak zor ayrıştığı ve daha düşük arıtma verimleri ile arıtıldığı belirlenmiştir. BTEX bileşenleri içerisinde toluenin sistem işletim parametreleri değişimlerine en hasssas madde olduğu ve bu değişimler sonucunda arıtma veriminin düştüğü tespit edilmiştir. Bu çalışma BTEX bileşenlerinin anoksik koşullar altında biyolojik olarak arıtıldığı ilk çalışma olmuştur ve ancak proses optimizasyonu için bundan sonraki çalışmalarda işletim koşullarında yapılacak iyileştirmelere ihtiyaç duyulmaktadır.

1. INTRODUCTION

Odor is typically defined as the “perception of smell” or in scientific terms as “a perception resulting from the reception of stimulus by the olfactory sensory system”. Unlike conventional air pollutants, odorous pollutants have different characteristics and their impact are tightly determined by human perception. Due to the subjective nature of odorous pollutant perception, odor pollution has become nowadays a topic of increasing importance within the global quest to improve air quality standards and increase well-being level of people. The increasing industrialization and encroachment of residencial areas in industrial sectors have resulted in an increase in the number of public complaints related to odor nuisance. Many studies have consistently pointed out the serious adserve effects of odorous emissions on human health and the environment, which resulted a increasing public concern to reduce the impact of odour pollution in cities. In order to find the proper strategy to prevent and to reduce the adverse effects of odor emissions, the identification of the problem via a proper characterization methodology followed by a selection of cost-effective and environmental friendly solutions to abate odor emissions should be investigated in the industrial sector. Among the industrial processes, food fermentation, and chemistry/petrochemical industries represent key pollution sectors due to their large volumes of odor emissions to atmosphere. This is central in many developing countries, where a major part of these industrial processes release their off-gases without any treatment. Apart from the above mentioned odour nuisance, waste gas streams released to atmosphere by chemical/petrochemical industries containg toxic volatile organic compounds such as ethanethiol, benzene, toluene, ethlybenzene and xylene (BTEX), which are responsible for health problems such as central nervous system depression, negative effects on the respiratory system and leukemia cancer. In this context, odor emissions abatement has become a priority that requires simultaneously a proper emission characterization and the development of cost-competitive and environmentally friendly abatement technologies.

1.1 Purpose of Thesis

It is generally accepted that for an effective and proper odor abatement to be implemented, the problem must first be quantified. Up to date, many studies didn’t take into account the characteristics of the emission, which resulted in a poor abatement performance. Within the primary aim of this thesis work, malodourous gas emissions in model industrial sectors such as food fermentation and chemical/petrochemical were investigated to obtain a proper characterization of the odorous waste gas. The second major goal of thesis work involved the development of cost-effective and green treatment technologies in the model industrial sectors. Until now many physical/chemical odor treatment techniques have been widely used due to their low footprint, high efficiency and rapid start-up, and extensive experience in design and operation, but these techniques are not environmental friendly and entail high investment costs. In this context, the specific aims of this thesis can be summarized as follows:

On this way specific aims of this study can be summarized as;

- Determination of odor threshold levels in food fermentation process with using both subjective and objective odor determination measurement “dynamic olfactometer” and “instrumental analyses” and investigation of the relationship between analytical measurement and olfactometric measurement.
- Semi-Pilot Scale Biofilter operation for biotreatment of well-characterized fermentation process emissions and investigation the influence of single and triplet compound feeding regime over biodegradation
- Lab scale anoxic biotricklingfilter operation for biotreatment of BTEX mixture and study of the influence of operation parameters.
- Kinetic Parameters estimation of petroleum industry emissions BTEX under O_2-free Conditions for single, dual and quaternary BTEX mixtures. and Lab Scale anoxic biotrickling filter operation for biotreatment of BTEX mixture and Study
- Feasibility of simultaneous use of microorganisms and algea for trimethylamine oxidaton and use of by-products for algal production

1.2 Scope of Thesis

Instrumental and olfactometric analyses are recommended as methods for odor nuisance assessment. Within instrumental techniques, gas chromatography coupled with mass-spectrometry (GC–MS) allows a qualitative and quantitative determination of the composition of a gas mixture, while sensorial techniques such as dynamic olfactometry uses human nose as a sensor to determine both the odor threshold and concentration/intensity of odor emissions.

This thesis was carried out in two universities. Thus, studies dealing with fermentation process emissions were carried out at Istanbul Technical University, where the characterization of odor emissions in bakery yeast fermentation process was conducted using instrumental and olfactometric techniques (Chapter 3). The performance of a semi-pilot scale biofilter for the treatment of single and terciary mixtures of gas pollutants from fermentation processes was also evaluated (Chapter 3). On the other hand, studies at Valladolid University were focused on the chemical/petrochemical industry as a model sector. Kinetic parameter estimation for microbial communities degrading O_2-free BTEX emissions under anoxic conditions were done for single, dual and quarternary mixtures (Chapter 4). In addition, the continuous biotreatment of BTEX emissions was carried out in an anoxic biotrickling filter for different operation conditions to reach best elimination of BTEX emissions (Chapter 5).

2. LITERATURE REVIEW

2.1 Air Pollution

Air pollution is defined as the presence of one or more substances in the air for a duration or at a concentration above their natural levels, with the potential to produce an adverse effect on humans and/or the environment (Seinfeld JH, 2006). It is considered nowadays one of the greatest threats for the environment and human health (Rene et al., 2011), not only due to the advances in science and technology in the last century that have led to incredible improvements in the quality of life, but also to the increased awareness of the society on the real impact of anthropogenic atmospheric pollution. However, air pollution has a long and evolving history in line with technological, political and economic changes. One of the major environmental air pollution disasters occurred in London on December 1952 when a thick layer of smog covered the city area during four days and resulted in more than 4000 deaths. The detrimental effects of the smog were associated with another 12,000 deceases that occurred two months following the event (Estrada, 2014). This episode is considered a turning point in the way air pollution was handled all over the world. After this big tragedy, many scientist focused on highlighting the detrimental effects of air pollution on human and as well as on environment. In addition, The World Health Organisation (WHO) has classified air pollution as the greatest environmental risk to human health in the present century (note that this is based on current risk, while longer-term environmental threats such as climate change, may exceed this in the future). Moreover, air pollution was reported in 2012 as the eight leading cause of death all over the world, with 4.2 million premature deaths globally being associated with ambient air pollution. Additionally, air pollution is considered as the primary cause responsible for many diseases such as stroke, heart disease, chronic obstructive lung diseases like lung cancer, asthma and pulmonary disease, diabetes and dementia, and acute respiratory infections in children (Barnett et al., 2005; Garcia et al., 2016; Huang et al., 2018; Li et al., 2017). In recent years, more than 90% of the world’s population is living in areas where air quality levels failed to meet the targets of WHO (Brugha et al., 2018). Apart from the adverse health effected of air pollutants, increased wellbeing standards and public awareness on the exposure to unpleasant air pollutants are leading to an intensification of complaints in residential areas.

Air pollutants include many different compounds from particles to gases, and are emitted from a wide range of sources including nature (volcanoes, forests, fires, etc.) and anthropogenic activities. While natural sources play an important role in local air pollution in arid regions more prone to forest fires and dust storms, the contribution from human activities far exceeds natural sources.

In this sense, human activities that are major sources of outdoor air pollution include Figure 2.1(Singh, 2017):

- Fuel combustion from motor vehicles (e.g. cars and heavy-duty vehicles)
- Heat and power generation (e.g. oil and coal power plants and boilers)
- Industrial facilities (e.g. manufacturing factories, mines, and oil refineries)
- Municipal and agricultural waste sites and waste incineration/burning
- Residential cooking, heating, and lighting with polluting fuels

Apart from that poor urban planning, which leads to sprawl and over-dependence on private transport, is also a key factor in the accelerated increase in pollution emissions.

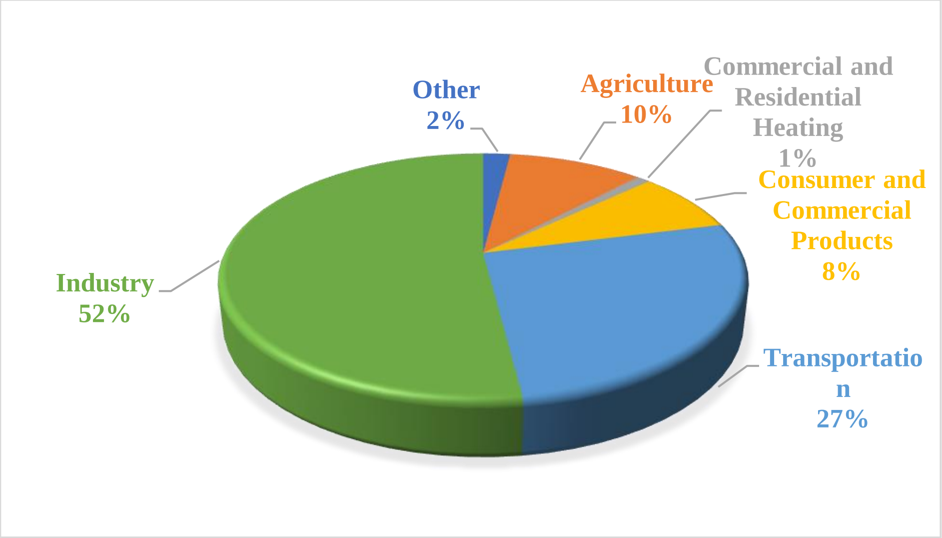

Figure 2.1 : Main anthropogenic sources of air pollution emissions (Singh, 2017)

All the above mentioned issues make atmospheric pollution control a key concern especially in the fast-developing urban areas driven by globalization and exponential economic growth.

2.1.1 Volatile organic compounds (VOC)

Volatile Volatile organic compounds (VOCs) are considered one of the main types of air pollutants due to their adverse effects on human health. The VOCs are organic chemicals with a boiling point below or equal to 250 ºC measured at a standard atmospheric pressure of 101.3 kPa, and include a wide range of chemical families such as aromatics, alcohols, alkanes, esters, aromatics, halocarbons, esters, ketones etc. VOCs are emitted to the atmosphere either in gas or liquid form from different sources: storage and transfer operations, waste disposal processes, and many industries such as paper, paint, solvents or fuel/petroleum industries (Balasubramanian et al., 2012; Yang et al., 2018). VOCs have become an important topic of research due to their complexity and important negative effects on human health (headaches, irritation, insomnia, loss of appetite, respiratory problems, damage to liver, kidney and central nervous system, etc.) and the environment (for instance in ozone formation)(Malakar et al., 2017; Wang et al., 2018). Apart from the detrimental health effects of VOCs, most of them are malodorous and can be perceived by the sense of smell despite being present at trace level concentration, potentially resulting in odor nuisanse on the nearby populations (Muñoz et al., 2010; Schiavon et al., 2017).

2.1.2 Odor

Nowadays odor generally defined as complex mixtures of multiple chemicals present at trace level concentrations that can be perceived by the sense of smell and its perception concentration determined in terms of odor unit which is dimensionless number where a panel is presented odor in decreasing dilution until it is detected. Odor nuisance is considered nowadays one of the most important environmental pollution problems due to its direct impact on the community's quality of life. Among all the senses, the sense of smell is considered the most complex due to a series of chemical and neurological interactions that take place in the human olfactory system, which hinders the proper understanding of odorous pollution and its impact on the population. Moreover, the subjective nature of odorous nuisance depends on the age, natural sensitivity, sex, personal experience, health problems, education, etc. (Bianchi et al., 2013; Sironi et al., 2010). Odor nuisance is also determined by odor characterisation parameters such as intensity, frequency and hedonic tone. Despite not being a direct cause of disease, long-term exposure to odorant emissions has a detrimental effect on human health and results in social and physical problems.

Odorous emissions are complex mixtures of multiple chemicals present at trace level concentrations (ppb-ppm range). Nevertheless, despite their low concentrations, the olfactory detection thresholds of these odorants determine the high impact associated to the odorous emission Table 2.1. The wide range of odorants include organic and inorganic compounds that are released to the atmosphere from various industrial activities in gaseous or particulate (dissolved in liquid drops) forms. Because of their structures, these compounds rapidly disperse in the surrounding environment leading to important disturbances. Furthermore, organic compounds might trigger ozone synthesis in the atmosphere and contribute to the formation of greenhouse gases with their oxidation products. Therefore, odor causing VOC emissions do not just threat human health and welfare (long-term exposure to such odorants cause nausea, headaches, insomnia, loss of appetite, respiratory problems, etc.), but also to air quality, as they contribute to photochemical smog formation and the emission of particulate secondary contaminants.

In recent days, attention to odorous VOCs has been growing because of the encroachment of residential areas on industrial activites (resulting in an increase in the number and severity of public complaints), and the awareness of people's need for a cleaner environment (Estrada et al., 2011). This has triggered governmental authorities to enforce stricter environmental regulations on odor emissions management, which has turned odorants abatement into a key issue for industrial operators (Badach et al., 2018; Capelli et al., 2008; Hayes et al., 2017). Basic legal requirements of European Union for ambient air quality control are specified in the Directive 2008/50/EC (2008) and Directive 2010/75/EU (2010). Also, Turkey enforced in 2013 the "Regulation on Control of Emissions that Contribute to Odor (KOEKHY)" to establish the legal requirements.

Table 2.1 : Olfactory detection threshold of most important malodorus compounds.

COMPOUND	DETECTION THRESHOLD (PPM)
H_2S	0.0005
DIMETHYL SULPHIDE	0.001
DIMETHYL DISULPHIDE	0.000026
AMMONIA	0.038
INDOLE	0.0001
TRIMETHYLAMINE	0.004
PROPIONIC ACID	0.028
BUTYRIC ACID	0.0003
BUTANONE	0.25
ACETADEHYDE	0.0001
TOLUENE	2.1
PHENOL	46

2.1.3 Kinetic parameter esmitation and modelling

The estimation of the kinetic parameters is essential in the field of biodegradation (Trigueros et al., 2010). Up to date, the biodegradation pathways for individual VOCs are well known and have been applied in the modelling field. However only few studies have focused on the assessment of substrate interactions such as inhibition, cometabolism and competition in VOCs mixtures (Akmirza et al., 2016; Deeb and Alvarez-Cohen, 1999; Horn and Jones, 1940). Particularly in the petroleum industry, the presence of several VOCs in gas emissions (i.e. mixtures of benzene, toluene, ethyl-benzene and xylene, BTEX) might result in positive or negative interactions during the biodegradation process, thus identifying and predicting this behavior is of key importance (Morlett-Chávez et al., 2010). The identification of the unpredictable behavior of multiple compound interactions during their biodegradation is possible with the estimation of kinetic parameters, which are playing a fundamental role for the optimization of planning, modelling, design management and upgrading of the biological systems and also usually a cheaper and faster tool to evaluate the impacts of changes in the operating conditions on system performance Furthermore, none of the studies available in literature have investigated up to date the kinetics of VOC biodegradation under O_2-free biodegradation processes for model sector emissions. (Akmirza et al., 2016). At this point, the determination of kinetic parameters under anoxic conditions will help to overcome the limitations to understand the biodegradation pathways and promote further studies to properly design and model biodegradation processes in petrochemical industry (Annesini et al., 2014)(Novaes et al., 2017).

Up to date, many different mathematical models have been used to estimate kinetic parameters, which were further used in optimizing bioreactor designs. Among the existing models, Monod model is widely accepted and applied as the most suitable model for kinetic parameter estimation under non-inhibitory conditions. Monod model offers a suitable estimation when describing the biodegradation of a single substrate, and parameters such as μ_{max} (the maximum specific growth rate), S (the concentration of the limiting growth substrate) and K_S (the half-velocity constant) can be determined with high accuracy in equation 2.1. On the contrary, Monod Model presents important shortcomings when estimating kinetic parameters for multiple substrates due to substrate interactions (Dette et al., 2005).

$$\mu = \frac{\mu_{max} S}{K_s + S} \tag{2.1}$$

To overcome this uncertainty, non linear regression models such as the Modified Gompertz kinetic model have been proposed for multiple compounds, where the kinetic parameters such as the maximum rate of production (R_m), the maximum production (P_{max}), the elapsed time (t) and the lag-phase (l), can be estimated with high certanity presented in equation 2.2. (Akmirza et al., 2016).

$$P(t) = P_{max}\, exp\left(-exp\,\left(\frac{R_m}{D_{max}}(\lambda - t) + 1\right)\right) \tag{2.2}$$

Nevertheless, further research is required for the optimization of such models, and only few studies have addressed the detailed mathematical modeling of multiple substrates biodegradation under anoxic conditions.

2.1.4 Industrial air pollution control

The proximity of residential areas to industrial facilities has increased the demand for VOC and odor characterization and control systems to a provide a clean and nuisance-free air (Mudliar et al., 2010). Industrial activities release gas pollutants into the atmosphere directly from their production prosesses or from failures or inadequacies on their installations. Meeting legal requirements on odor-causing VOC emissions and increasing quality of life makes the management of these emissions a mandatory and complex issue (Barbusinski et al., 2017a). For an effective and proper odor nuisance abatement to be implemented, the problem must be first quantified, and the characteristics of the waste gas stream should be identified prior selection of optimum VOC treatment technology (Romain et al., 2008).

Due to the challenging character of odorous VOC emissions, both instrumental and sensorial analyses are recommended for a complete chemical characterization of the emission together with the evaluation of odor annoyance (Wenjing et al., 2015). In this sense, instrumental techniques can only assess the quantitative composition of a gas mixture, while sensorial analysis is mandantory to determine the intensity of odor emissions and its impact on the receptor (Capelli et al., 2008; Muñoz et al., 2010).

In waste gas streams, odorants and VOCs are usually present at very low concentrations, thus preliminary adsorption (concentration) on sorbent tubes followed by gas chramography coupled with mass spectrometry is typically for characterization. Method EPA TO-17 is officially announced by the US-EPA as the preferred analytical method. On the other hand, dynamic olfactometry, which allows to characterize the direct effect of the odorous VOC emission on people and eliminates the lack of subjectivity of the instrumental analyses, is the most common sensorial technique (Capelli et al., 2013).

2.1.5 Air pollution control technologies (VOC and Odor Emissions)

Due to negative impact of malodorous VOC emissions on human and environment, there is an urgent need to develop cost-effective VOC and odor control systems to ensure a proper air quality (Mudliar et al., 2010). Also governmental authorities urge polluting industries to adopt proper treatment processes to overcome population complaints and impose penalties when industrial facilities exceed regulatory limits. Despite the paramount technological breakthroughs carried out during the last three decades, it is still air pollution treatment systems are limited by the financial cost and low waste gas treatment efficiency when multiple compounds are responsible for air pollution.

The selection of proper treatment processes mainly depends on the composition and flow rate of the waste gas stream, odor emission characterization being one of the key challenges during technology selection. A propoer odor emission characterization helped the authorities to select the most suitable odor treatment process to tackle the odor problem. In addition, other parameters such as moisture, temperature and particulate content have to be taken into account to properly select, design and operate gas treatment technologies. Furthermore, the local conditions, availability of land area, and limitations in investment and operating cost are considered critical parameters to be considered. In this way, selection and design of proper odorous VOC removal processes must aim at finding an optimum where the required treatment efficiency is achieved as cost-effectively as possible, while offering environmentally friendly alternatives that can be adapted to the specific conditions (Schlegelmilch et al., 2005).

Nowadays, a large variety of alternatives used for off-gas treatment is based on pollutant transfer from the waste gas stream towards a solid (adsorption) or a liquid (absorption) phase, commonly followed by a biological or chemical oxidation of the pollutant (Estrada, 2014). During the past decades, physical/chemical treatment processes such as condensation, incineration, adsorption, thermal oxidation, chemical scrubbers, ozonation and catalytic oxidation have been widely used due to their advantages: rapid start-up, low footprint and widescale experience in design and operation. Among these technologies, chemical scrubbers and incineration processes represent nowadays a highly costly alternative for the treatment of odorous emissions both in terms of operation, due to their high chemical and heat demand (Gabriel and Deshusses, 2003). Other well-known technologies such as activated carbon adsorption or UV / ozone-based treatments do not often provide satisfactory levels of treatment because of the high moisture content typically present in odorous emissions. Apart from these drawbacks, their non-environmental friendly nature associated to the generation of secondary waste streams often limits their implementation in nuisance sensitive scenarios (Lebrero et al., 2010). In this context, the development of environmentally friendly and economically viable alternative treatment technologies has received increasing attention worldwide. Nowadays, biological air treatment has emerged as a cost-efficient and environmentally friendly alternative to physical/chemical odor treatment technologies. It relies on the activity of microorganisms able to biodegrade organic contaminants for cell growth and maintenance, with a conversion into non-odorous and less harmful by-products (mainly CO_2 and H_2O) (López et al., 2018; Malakar et al., 2017b; Schlegelmilch et al., 2005). Additionaly, the low pollutants concentration found in odorous waste gas streams and the relatively high flow rates constitutes the idel framework for the application of biotechnologies illustrated in Figure 2.2. As a result of these advantages, within the last decades biological techniques have been applied more frequently as promising alternatives to control odorous VOC emissions (Balasubramanian et al., 2012b; Van Groenestijn and Kraakman, 2005). Though a number of different bioreactor configurations have been used up to date, the main types of conventional air-phase bioreactors include biofilter (BF), bioscrubber (BS), biotrickling filter (BTF), and bubble column bioreactors (BCB). The main differences between these reactor configurations are related with the type of liquid (continuous flow or stationary) and the microbial community (suspended in the liquid broth or fix forming a biofilm) (Wu et al., 2018).

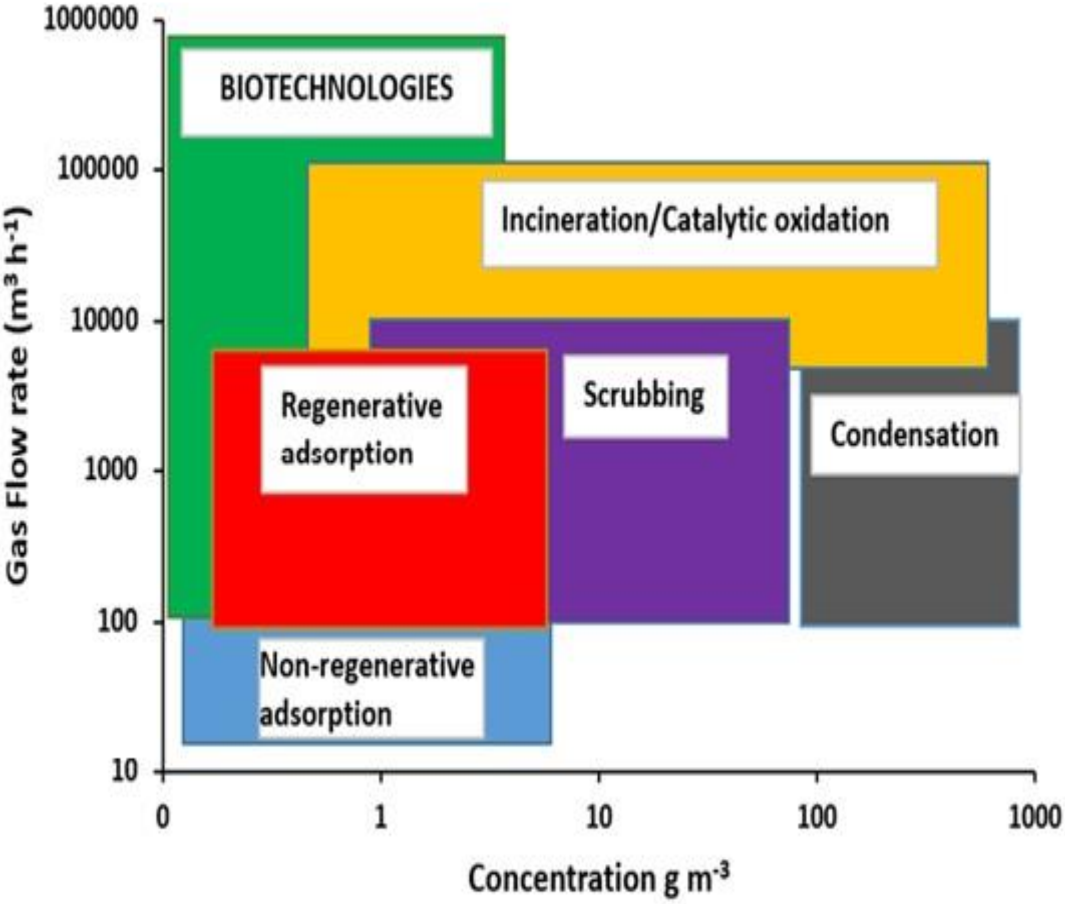

Figure 2.2 : Comparative chart for the selection of air pollution control techniques traditionally employed since the 90s.

2.1.5.1 Biofilters

Biofilters are most popular bioreactor type capable of removing a wide range of organic and inorganic pollutants at high removal efficiencies (>90%). In general, biofilters are defined as bioreactors in which a waste gas stream is passed through a porous packed bed material on which a mixed culture of pollutant-degrading microorganisms are immobilized. Compost, peat, root wood, bark, wood chips and their different kind of combinatios are typically used as packed material in the biofilter columns and also responsible to supply essential nutrient for microbial growth (Barbusinski et al., 2017b). BFs posses a wide-range of application and up to now have been used in Europe more than 600 chemical processing industries to treat VOC and odor exhaust streams (Gallastegui et al., 2011). Although BFs first application was devoted to the treatment of odorous emissions from sewage treatment plants and composting facilities, BFs now find wide application in the treatment of several VOCs and odors such as ammonia, hydrogen sulphide, mercaptan, disulphides, etc., and VOCs like propane, butane, styrene, phenols, ethylene chloride, methanol, etc.

Overall, BFs have advantages over other biological treatment systems due to their easy start-up procedure, cost effectiveness in both capital and operation cost, low-pressure drops and no generation of secondary waste gas strems. Additionaly BFs are used for the treatment of large volumes of air streams containing low concentration of VOCs or odorants, and recent studies have shown that 60 out of 189 hazardous air pollutants (HAPs) can be successfully treated with biofilters (Shareefdeen et al., 2005). Biofilters have high removal efficiency for hydrophilic compounds and up to 99 % removal was observed VOCs (Henry's Law constant lower than 0.07 M atm^{-1}) while moderately efficent and resulted a removal up to 75 % for hydrophobic compounds (up to 75%) (Henry's Law constant higher than 20 M atm^{-1}) (Estrada et al., 2011) due to limitations by mass transfer.

Despite the above cited advantages of biofilter systems, they have some drawbacks during operation such as the difficulty to control moisture and pH in the biofilm, clogging of the medium due to particulate matter and biomass overgrowth, problem of packing media deterioration and lower removal efficiencies at high VOC concentrations (Mudliar et al., 2010). Schematic presentation of biofilter setup has been illustrated in Figure 2.3.

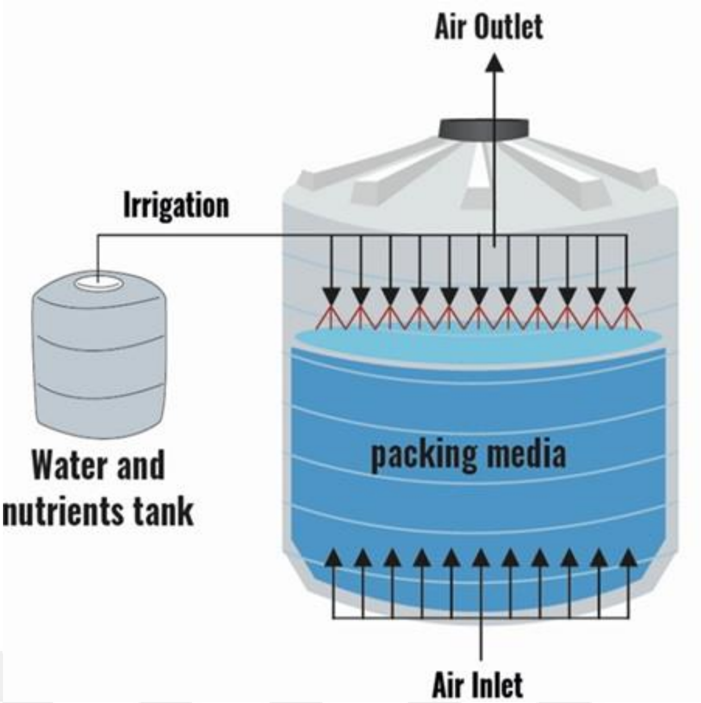

Figure 2.3 : Schematic view of biofilter (Estrada, 2014).

2.1.5.2 Bioscrubber

Bioscrubbers consist of two subunits: a packed tower (absorption column) and a bioreactor, that seperate gas absorption and biodegradation processes Figure 2.4. In bioscrubbers, the input waste gas enters first in the absorption unit where the pollutants are transferred from the gas phase to an aqueous solution (flowing either co-currently or counter-currently) (Nisola et al., 2009; Potivichayanon et al., 2006). The liquid scrubbing solution containing the dissolved gas contaminants is subsequently drawn off and transferred to a biological tank that contains the microorganisms, generally suspended in the cultivation broth, responsible for the biodegradation of the absorbed gas. Bioscrubbers are the preferred technique to treat hydrophilic VOCs (alcohols, ketones), with low Henry coefficients (<0.01), since they must be transferred to the aqueous phase prior to degradation.

Main advantages of bioscrubbers (Mudliar et al., 2010):

- Operational stability and better control over operating parameters such as pH, moisture and nutrient content.
- Relatively lower pressure drop and less area requirement apart biotreatment alternatives

Disadvantages of bioscrubbers include:

- Low removal efficiency for hydrophobic compounds.
- Provides low specific surface area for gas–liquid mass transfer (generally <300 $m^2 m^3$)
- Excess sludge generation
- Generation of secondary liquid waste stream

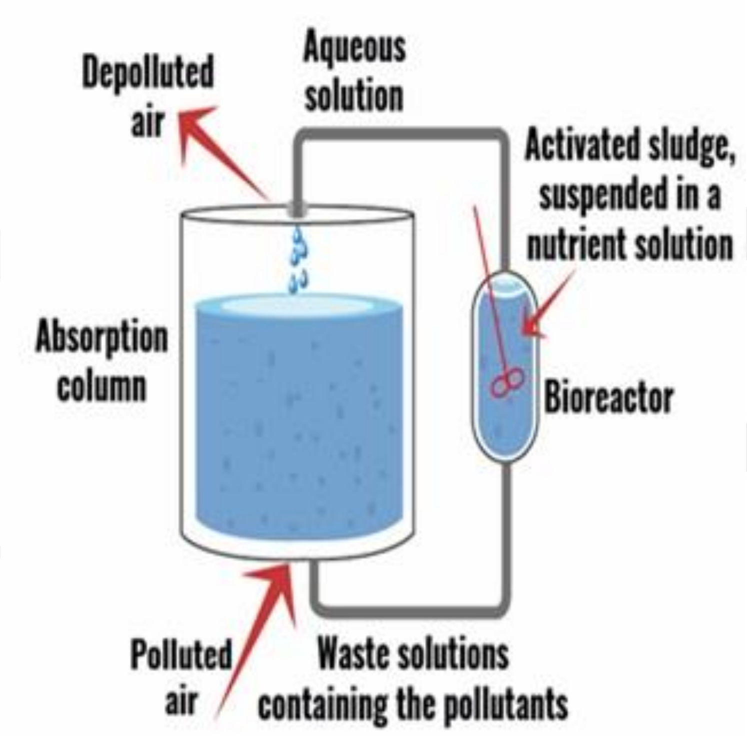

Figure 2.4 : Schematic view of bioscrubber adapted (Mudliar et al., 2010).

2.1.5.3 Biotrickling filter

A biotrickling filter (BTF) consists of a cylindrical column packed with inert material which promotes the growth of microorganisms (Estrada, 2014). The polluted gas stream passes through the packed bed, which is continuously irrigated with a recirculating aqueous solution containing essential nutrients required by the microbial community, washing away the excess biomass and secondary products (Mudliar et al., 2010; Schiavon et al., 2016). In BTFs, the microorganisms grow forming biofilms on the surface of the packing material, and pollutants are initially absorbed in the aqueous film trickling over the biofilm and degraded afterwards by the microbial community and shown in Figure 2.5. BTFs present multiple advantages over other biological alternatives, such as lower pressure drops across the packed bed due to the high porosity of the inert packing material, less operating costs, easier control of the operating parameters (pH, nutrient feed), capability to treat by-products from VOCs degradation, lower area requirements, less dead zones, and higher removal efficiencies (REs) for aromatics, chlorinated hydrocarbons and organic sulfur compounds (Akmirza et al., 2017; Mudliar et al., 2010). Despite these advantages, the accumulation of excess biomass in the filter bed is considered the major drawback of BTFs.

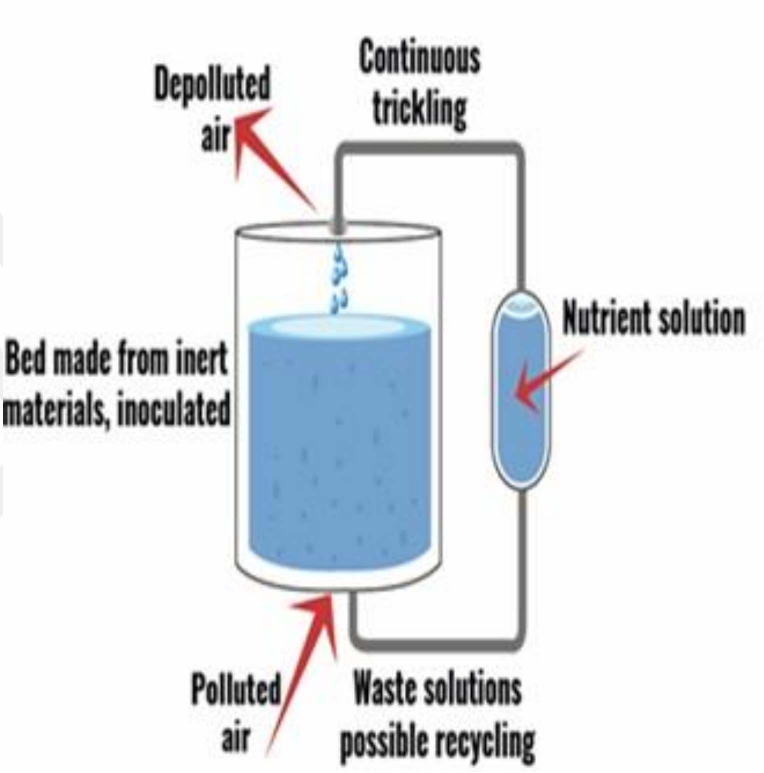

Figure 2.5 : Schematic view of biotrickling filter (Adapted (Mudliar et al., 2010)).

2.1.5.4 Bubble column bioreactor

The VOCs removal performance in the aboved-revised biotechnologies is usually limited by mass transfer of the compound from the gas to the liquid phase or biofilm, thus resulting in low removal efficiencies for poorly water-soluble (hydrophobic) compounds. In this context, bubble column bioreactors (BCBs) present high mass transfer capacities compared with other biotreatment alternatives due to the supplementation of the gas stream through ultrafine bubble diffusers with micropores< 0.5 µm, which allow increasing the contact between the gas and liquid phase, and results in an enhanced mass transfer. Generally, BCBs consist of cylindrical vessels with a gas distributor at the bottom where it is illutrated in Figure 2.6. The gas stream is sparged in the form of fine bubbles into the biomass suspension. In addition, the performance of BCBs can be further boosted via internal gas recycling, which allows the decoupling of the actual gas residence time and turbulence in the microbial broth from the overall empty bed residence time (Ahmed et al., 2010; García-Pérez et al., 2018; Kantarci et al., 2005). Additionaly, BCBs require little maintenance, present low footprint and have low operating costs compared to other reactor configurations. Due to these advantages, BCBs are widely employed in chemical, biochemical and petrochemical industries.

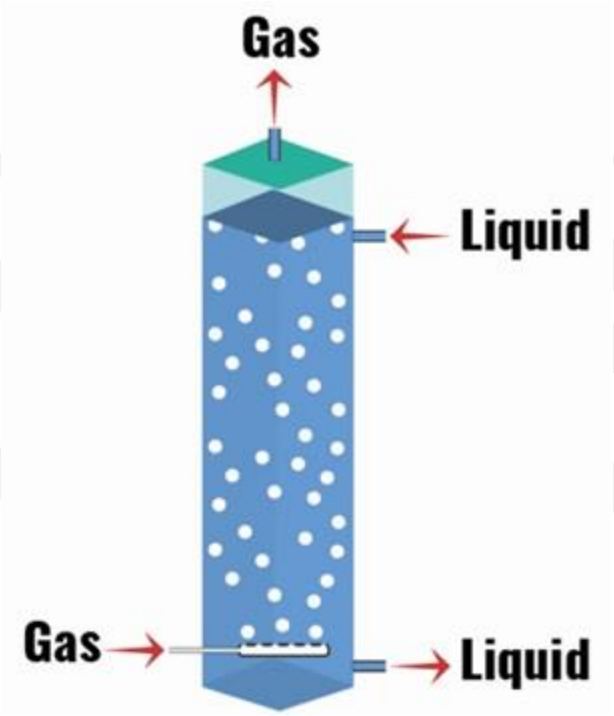

Figure 2.6 : Schematic view of bubble column bioreactor (Sui and Sakarinen, 2017).

BCBs can easily adapted to photobioreactors, which are bioreactors that utilize a light source to promote the phototrophic growth of algae and cyanobacteria in symbiosis with bacteria. Photobioreactors are typically described as an enclosed, illuminated culture vessel designed for controlled biomass production (Singh and Sharma, 2012). Photobioreactor support the symbiotic growth of microalgae and bacterial, a consortium that provides mutual benefits to remove malodorous compounds such as amines due to their superior NH_3 fixation and enhanced O_2 supply.

2.1.6 Model sectors

From the beginning of the industrial revolution in the mid-1900s, industries started to release air emissions to atmosphere without considering protection to the environment or human health. More recently, many different types of industries and related sectors have been required to meet higher social expectations in the form of new laws and regulations. Because of these new air quality standards, especially focused on odorous VOCs, industries started to control their gas emission via improving process efficiency and implementing end-of-the-pipe treatment processes. Among them, bakery yeast production process represent one of the main industrial sources of odors due to its huge VOC relase from its fermentation cycle where general cycle is illustrated in Figure 2.7. Ethanol and acetaldehyde, which are VOCs with low odor threshold levels (5-500 ppbV and 10-120 ppbV for ethanol and acetaldehyde, respectively), are released into the ambient air from the process, resulting in important odor nuisance in the surrounding neigbourhoods. Besides the main air emissions of acetaldehyde and ethanol, many different types of VOCs are relased to the atmosphere via yeast fermentation process, however the detailed waste gas characterization is still unknown.

The organic chemistry production industry was intestigated as another model sector due to the large variety of chemical compound manufacture, which included raw compounds that used frequently in petrochemical plants. Among malodorous compounds, trimethylamine (TMA, C3H9N) has been identified as one of the main pollutants emitted: it is a potentially toxic and likely carcinogenic VOC with a low odor threshold level of 0.2 µg m-3 (Aguirre et al., 2018; Chang et al., 2004; Wan et al., 2011). Proper management of TMA emissions according to legislation limits is crucial to not only avoid health hazards and ensure occupational safety, but also to reduce impacts on the environment (Chang et al., 2004; Perillo and Rodríguez, 2016).

P.S. due to the ongoing work on TMA biodegradation, its not presented as part of the thesis work.

Petroleum industry is also an important source of VOCs emission, being benzene, toluene, and ethylbenzene and xylene (BTEX) crucial pollutants due to their wide range of use in the sector. In this sense, BTEX emissions accounts nowadays for up to 59% (w/w) of gasoline pollutants and represent 80% of the total VOC emissions in petrochemical plants (El-Naas et al., 2014; Gallastegui et al., 2011). In addition, BTEX have genotoxic properties and also listed in the Hazardous Air Pollutants and have the rank 78 in the Comprehensive Environmental Response, Compensation, and Liability Act (CERCLA) List from the 275 substances identified to pose most significant potential threat to human health (Akmirza et al., 2017; Carvajal et al., 2018) .

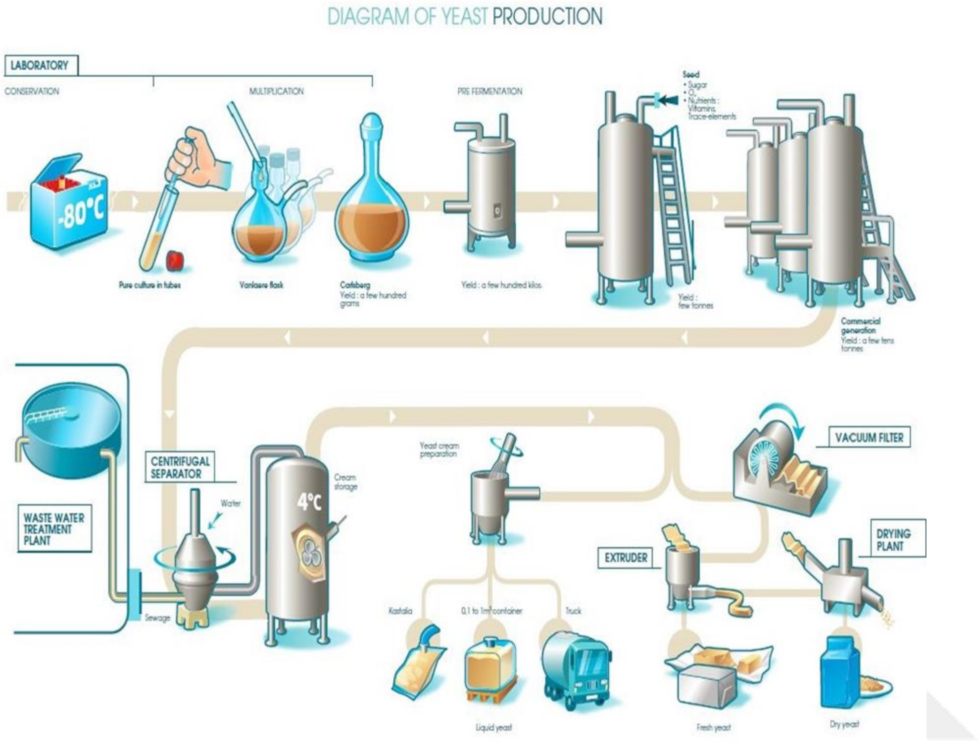

Figure 2.7 : Bakery yeast fermentation process flow chart.

3. YEAST PRODUCTION PROCESS EMISSION CHARACTERIZATION AND TREATMENT

Through various type of industries, bakery yeast production process come to forward due to realase of high frequency of malodorous emissions from its process to atmosphere that resulted odor nuisance at surrounding settlements. Consequently its important to identify the emission characterization in both objective and subjective viewpoint for the proper treatment technology selection . Within this chapter bakery yeast fermentation process was investigated in a key study to identify emission characterization and treatment of well-characterized process emissions with biological treatment of biofilter.

3.1 Yeast Production Process Description

Bakery yeast production factory which was surrounded with residental areas was selected and fermentation process waste gas characterization and use of biofilter application for selected and characterized waste gas stream in the yeast production process were studied.

3.2 Gas Sampling Methodology

Malodorous waste gas emissions in bakery yeast production process were characterized with both instrumental and olfactometric techniques to reveal all kind of aspect that resulted odor nuisance. Samples for instrumental analyses were carried out from the waste gas discharge point at the stack and to obtain proper data samples were taken hourly to simulate a complete fermentation cycle that lasting approximately 17 hours.

Active sampling procedure was followed according to EPA Standard Method TO17 *“Determination of Volatile Organic Compounds in Ambient Air Using Active Sampling Onto Sorbent Tubes”* which is registered as an official method. Within the procedure multisorbent tubes that contained the sorbent materials 20:35 Tenax-TA™, 60:80 Carboxen™ 1000, and 60:80 Carbosieve™ SIII were used to enlarge the scale of capture both hydrophobic and hydrophilic compounds onto surface of sorbent tubes. Triplicate sampling procedure was followed and collected samples were brought immidiately to the laboratory of Istanbul Technical Universiy Environmental Engineering Department and analyzed within 24 h, following ASTM 2009 standards. To reveal sensory effect of malodorous emissions on people dynamic olfactometry measurements were carried for the process emissions. According to first instrumental analyses results peak emission hours of the process were identified and were selected for the second characterization assay and dynamic olfactometry measurements were carried out according to EN 13725:2003 standards simultaneously with second instrumental measurements. Samples for dynamic olfactometry analyses were collected into tedlar bags with a volume of 1 Liter and were analyzed by accredited laboratories.

3.2.1 Instrumental analyses

The instrumental analyses for the sector emission chemical characterization y gas chromatograph (GC, Agilent 7890A, USA) equipped with a mass selective detector (Agilent 5975C, USA) was used following the method of EPA TO17 US-EPA, 1999a, Compendium Method TO17 (ASTM,2009). To desorb pollutants that were adsorbed on the surface of multisorbent tubes, Thermal Desorber (TD, CDS 9300) was used as preconcentration equipment and samples were injected primarly to TD unit and get concentrated for 10 minutes at 250^{o}C then the concentrated gas stream passed through the a HP5MS (30 m × 0.25 mm × 0.25 μm) GC column. Oven temperature initially maintained at 40^{o}C for 3 min, increased at 20 °C min^{-1} up to final temperature of 140 °C and hold for 3 min at 140°C. Process waste gas emissions in the samples were identified by comparing their retention times with the standards.

Thermal Desorber unit was discarded during the operation of pilot scale biofilter analyses which allowed to follow direct sampling. Samples from the inlet and outlet sampling port of the column were collected with using 250 μl gas-tight syringe (Hamilton, USA) and direct injection to GC/MS were done by using same instrumental method for chemical component characterization.

3.2.2 Dynamic olfactometric analysis

To understand sensorial impact of odor emissions dynamic olfactometry analyses were carried out parallel to instrumental analyses. Generally it is defined as sensorial technique directly related to the perception of human nose and allows to characterize odor concentration. At the previous studies of Sironi it is mentioned the number of dilutions of odorous gas sample with neutral air that required to bring the odorous sample to its odor detection threshold concentration and used as European odor unit per cubic meter (ouE m-3). In general analyses are depend on dilution of the sample until its threshold level, at that level the odor can be perceived by 50 % of the panel members. As a consequence of this concept gas samples from the fermentation process were taken with following the official sampling and analyse method of EN 13725 (2003) and samples were diretly send to accredited laboratory and analyses were done in the following 24 hours after sampling with using olfactometer ECOMA TO8 GmbH. During the dynamic olfactometry analyses panel members sniffed odorous gas samples with an increasing concentration until start to sense an odor that is different from the neutral reference air. Odor concentration(cod) was then calculated as the geometric mean of the odor threshold values of each panelist. Detailed dynamic olfactormetry analysis method was described by the authors elsewhere.

3.3 Biofilter Experimental Setup and Operation Procedure

Semi- pilot scale operation of biolfilter carried out at the laboratories of Istanbul Technical University Department of Environmental Engineering. The biofilter consisted a cylindrical jacketed PVC column with 0.2 m inner diameter and height of 2.7 m. To increase the superfacial area Rashing rings (specific surface area >400 m2/m3) were used as packing material and in a working volume of 47 Liter. Activated sludge obtained from recycling line from wastewater treatment plant of Ambarli, Istanbul (Turkey) was used as inoculum and schematic view of experimental setup is shown in Figure 3.1.

Incolumn was concentrated via following the procedure; 8 L of the activated sludge with a volatile suspended solids (VSS) concentration of 12500 mg L-1 were resuspended was settled in a one-litre cylinder. Subsequently, the supernatant was replaced with mineral salt medium (MSM) to rinse the sludge, periodically, in a batch pattern washed couple of times and filled up to 2 L with fresh MSM added to the biofilter column and homogenised before the start-up. The system operation was continued for 144 days within 6 different steady-state conditions and was presented in Table 3.1.

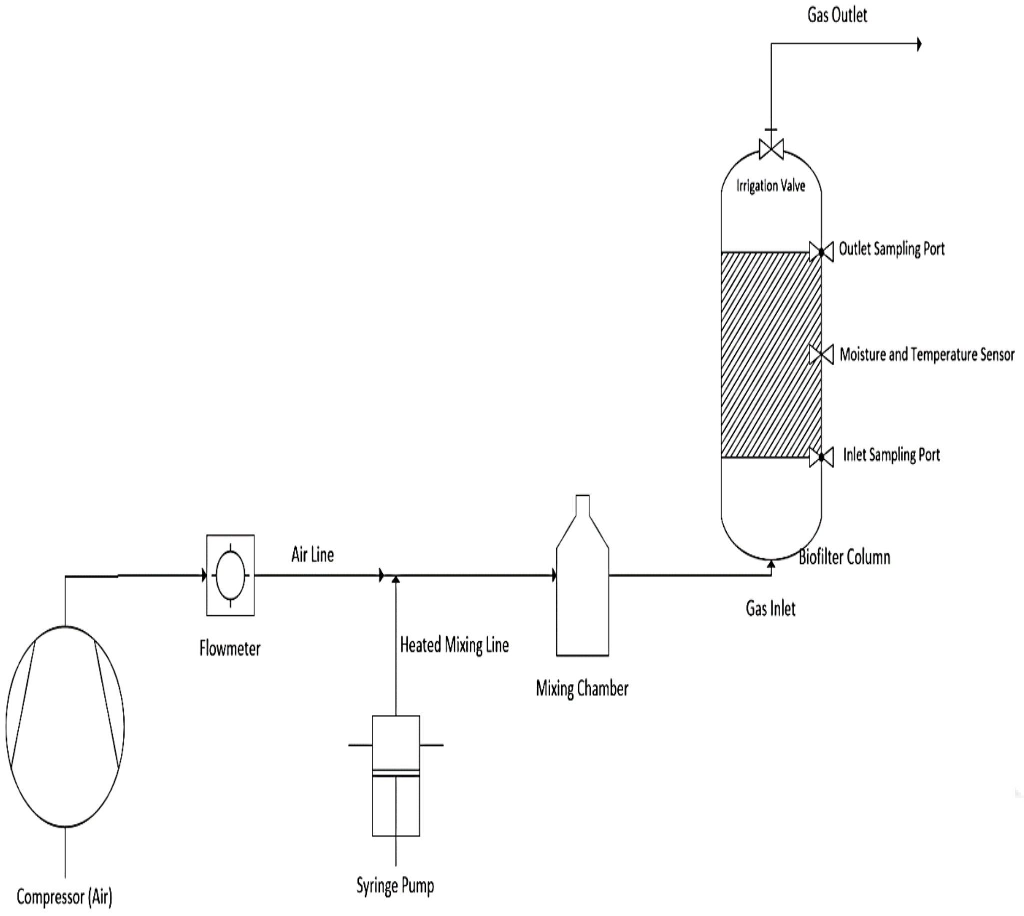

Figure 3.1 : Schematic representation of experimental setup.

Table 3.1 : Experimental conditions established during the operational stages.

Stage	**Days of Operation**	**Compound**	**Organic Load**
1	1-15	Ethanol	10.1 ± 2.3 g m^{-3} h^{-1}
2	15-30	Ethanol	16.5 ± 1.6 g m^{-3} h^{-1}
3	30-92	Ethanol	28.4 ± 5.5 g m^{-3} h^{-1}
4	92-122	Ethanol, Acetaldehyde and Acetone	41.27 ± 6.8 g m^{-3} h^{-1}
5	122-129	Ethanol, Acetaldehyde and Acetone	61.92 ± 8.8 g m^{-3} h^{-1}
6	129-144	Ethanol, Acetaldehyde and Acetone	41.27 ± 6.8 g m^{-3} h^{-1}

Due to high concentrations of ethanol(E) over other process emissions that was determined with instrumental analyses, start-up of biofilter operation was began primarily for single E removal and the E was injected to the synthetic waste gas stream in a liquid from the bottom of the reactor column in a counter mode with the humidification liquid flow for single component operation stages. The biofilter system was operated with a waste gas flowrate of 2 m^3 h^{-1} and that resulted gas loading rate of 63.7 m^3 m^{-2} h^{-1} consisting E emissions of 237 ± 55 mg m^{-3}, 387 ± 37 mg m^{-3}, 667 ± 131 mg m^{-3}, respectively during the first 92 days of operation. To simulate proper waste gas stream in the fermantation process acetaldehyde (AD) and acetone(A) were added to feed gas stream at the 93rd day of operation, , containing emission concentrations at inlet gas stream as 647 ± 97 mg m^{-3} for E, 255 ± 48 mg m^{-3} for AD and 68 ± 16 mg m^{-3} for A.

Same operation conditions were followed during the 4th stage of operation and EBRT maintained as 80 seconds until the 5th stage of operation where gas flow was increased to 3 m^3 h^{-1} which is resulted a decrease in EBRT to 53 seconds while gas loading rate increased to 95.5 m^3 m^{-2} h^{-1}. Irrigation liquid maintained moisture content approximately 50 %in biofilter column and also included all nutrients and was composed of (g L^{-1}): $Na_2HPO_4 \cdot 12H_2O$, 6.15; KH_2PO_4, 1.52; $MgSO_4 \cdot 7H_2O$, 0.2; $CaCl_2$, 0.038; and 10 mL L-1 of a trace element solution containing (g L^{-1}): EDTA, 0.5; $FeSO_4 \cdot 7H_2O$, 0.2; $ZnSO_4 \cdot 7H_2O$, 0.01; $MnCl_2 \cdot 4H_2O$, 0.003; H_3BO_3, 0.03; $CoCl_2 \cdot 6H_2O$, 0.02; $CuCl_2 \cdot 2H_2O$, 0.001; $NiCl_2 \cdot 6H_2O$, 0.002; $NaMoO_4 \cdot 2H_2O$, 0.003 (Muñoz et al., 2013).

Gas samples from inlet and outlet were collected with a gas-tight syringe (Hamilton, USA) and analysed at GC/MS and E, AD and A concentrations in the gas phase were determined. Liquid samples were daily analysed to determine the pH levels on the irrigation liquid. Moisture content in the biofilter coloumn was monitored continously via moisture meter and temperature keept mainted appro. 20 in the column and was monitored continusly via temperature probe.

Terms of the elimination capacity (EC) was used as evaluation parameter of the biofilter performance and presented in equation 3.1 which were calculated by following and

$$\text{Elimination Capacity (g m}^{-3}\text{h}^{-1}) = \frac{(Cin - Cout) * Qgas}{V} \tag{3.1}$$

C_{in} : Inlet Concentration (mg m^{-3})

C_{out}: Outlet Concentration(mg m^{-3})

Q_{gas} : Gas flowrate (m^3 h^{-1})

V: Volume of biofilter (m^3)

3.4 Results and Discussion

Analytical and dynamic olfactometric analyses results were presented in this section. Pilot scale biofilter operation for characterized waste gas stream in food fermentation process were carried out for 144 days.

3.4.1 Emission character determination via TD-GC-MS

Instrumental analyses were carried out with TD-GC–MS to determine chemical composition of entire food fermentation cycle process. To understand chemical composition and sensorial effects of waste gas emissions on people at the same time, intrumental analyses were repeatad simultaneously to dynamic olfacometry measurements. With the aim of first test assay peak hour emissions were determined and selected for following olfactometric analyses and at the second assay, samples on sorbent tubes were collected hourly as duplicate, simultaneous to dynamic olfactometry measurements and results were illustrated in Table 3.2 and

Figure 3.2 In accordance with the results E and AD were found as primary pollutants that were consisting in total higher than 95% of emissions with the maximum concentration of 764 and 331 mg m^{-3}, respectively while only less on the opposite only less than 5% of total emissions were relased to atmosphere as A with the maximum concentration of 65 mg m^{-3} and in addition trace propanol concentration was analysed during the first hours of fermentation cycle. Food fermentation process emissions reached to maximum after the start-up of the cycle and continued during the first half of the process until the 7th hour of cycle and after the first half emissions were started to drop clearly after the 8th hour of process and only trace emissions were observed at the last 3 hour of the cycle.

Table 3.2 : Time course of average food fermentation cycle emissions.

Fermentation Cycle (h)	Ethanol (mg/m^3)	Acetone (mg/m^3)	Propanol (mg/m^3)	Acetaldehyde (mg/m^3)	Total Emission(mg/m^3)
1	764	65	21	331	1181
2	711	55	18	285	1069
3	704	54	20	275	1053
4	720	58	21	290	1089
5	634	44	18	215	911
6	629	38	18	197	882
7	720	47	20	224	1011
8	612	39	17	197	865
9	663	34	15	172	884
10	396	23	11	110	540
11	359	31	6	145	541
12	210	20	1	88	321
13	109	10	1	43	163
14	15	3	0	7	25
15	1	2	0	5	8
16	0	0	0	1	1
17	0	0	0	0	0

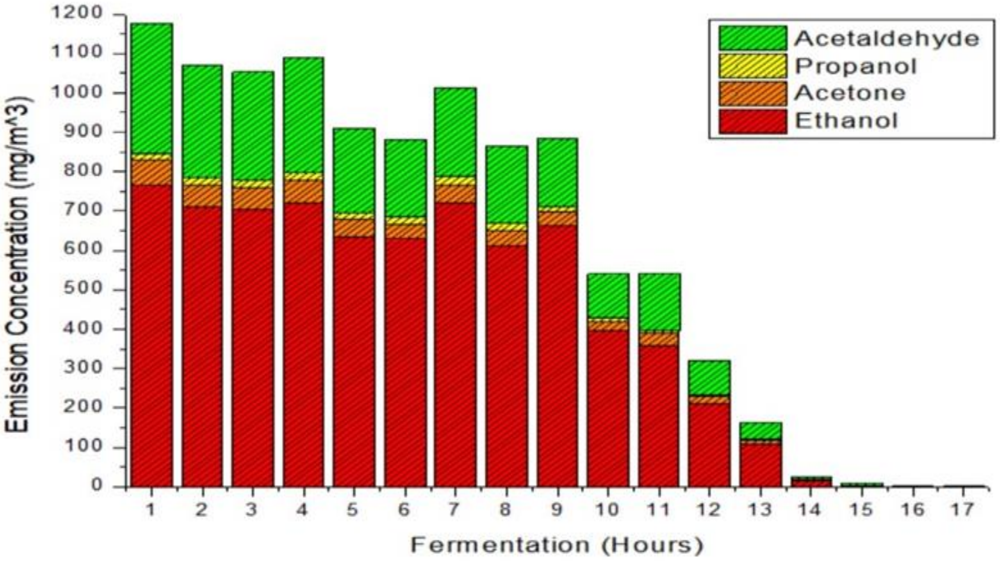

Figure 3.2 : Change of food fermentation process emissions versus time.

3.4.2 Dynamic olfactometry analyses and odor concentration determination

Peak hour emissions according to the first instrumental analyses were selected for dynamic olfactometry measurements and on the second sampling champain, the samples from 1st and 4th hour of process cycle were collected for dynamic olfactometry analyses while simultanouesly the second instrumental measurements were repeated for entire food fermentation cycle. Dynamic olfactometric measurements were analysed by sniffing the malodorous gas emissions by panel members and odor threshold level were perceived by panel members as 39725 OU/m^3 and 28090 OU/m^3 respectively, the first of which consituted nearly 40 times higher odor concentration than allowed to turkish legislation limits. Furthermore the results obtained form dynamic field olfactometry measurements matched with the previous odor threshold levels that mentinoed in literature (Leonardos et al., 1969) and Table 3.3 represents results for dynamic olfactometric measurement.

Table 3.3 : Dynamic olfactometry odor concentration.

Fermentation Cycle (h)	Sampling Point	Odour Concentration 1. Measurement	Odour Concentration 2. Measurement	Odour Concentration 3. Measurement	Odour Concentration Geometrical Mean
1	Fermentor Chimney	36781	41285	41285	39725
4	Fermentor Chimney	36781	21870	27554	28090

3.4.3 Biofilter operation results

Due to the presense of E as most extensive malodorous emission source in the process, biofilter operation was started first for single E feed with an empty bed residence time (EBRT) of 80 seconds and 237 ± 55 mg m^{-3} of E concentration and at an air flowrate of 2 m^3 h^{-1}. Within the startup period, the RE gradually increased to 90±5% at the 3rd day, corresponding to EC of 8.5 ±0.7 g m^{-3} h^{-1} that illustrated in Figure 3.3. During the first stage , the system reached to the RE up to 98±1% corresponding an E.C. of 9.5 ±0.7 g m^{-3} h^{-1} at the 12th day of the operation. At the day 15, second stage operation was started by increasing the inlet E concentration to 387 ± 37 mg m^{-3} where it didn't resulted a significant change on both RE and EC while biofilter was able to remove 95% of organic load input. At the third stage of operation by day 30, E concentration at the inlet increased was increased again two times and reached to 667 ± 131 mg m^{-3} that presented peak hour E emissions during fermentation cycle that was determined by instrumental analyses. A step change in E concentration was resulted in only slight reduction in RE as 90±1% with an EC of 28.3 ±1.1 g m^{-3} h^{-1} by day 33 while on the oncoming days biofilter recovered again itself again and RE of 97±1% with an E.C of 31.7 ±1.2 g m^{-3} h^{-1} was recorded by the day 54. By accident a problem at the irrigation line at the day 62th of operation caused a sharp decrease in RE to 81±2% corresponding to E.C of 23.3 ±1.0 g m^{-3} h^{-1} in consequence of decreasing the microbial activity under limited moisture content below % 25. At this situation, it is significant to notice previous authors were refered the contagion of moisture content reduction on microbial activity and studies reported a sharp drop off at RE when the moisture content recorded below % 35 (Xue et al., 2018; Yang et al., 2014). Considarable increase was recorded within reaching normal irrigation cycle to keep maintain the moisture content approximately % 50 and system recovered itself and maximum removal was noticed by the day 77 of operation with RE of 98±1 % and E.C. of 32.7 ±0.9 g m^{-3} h^{-1}.

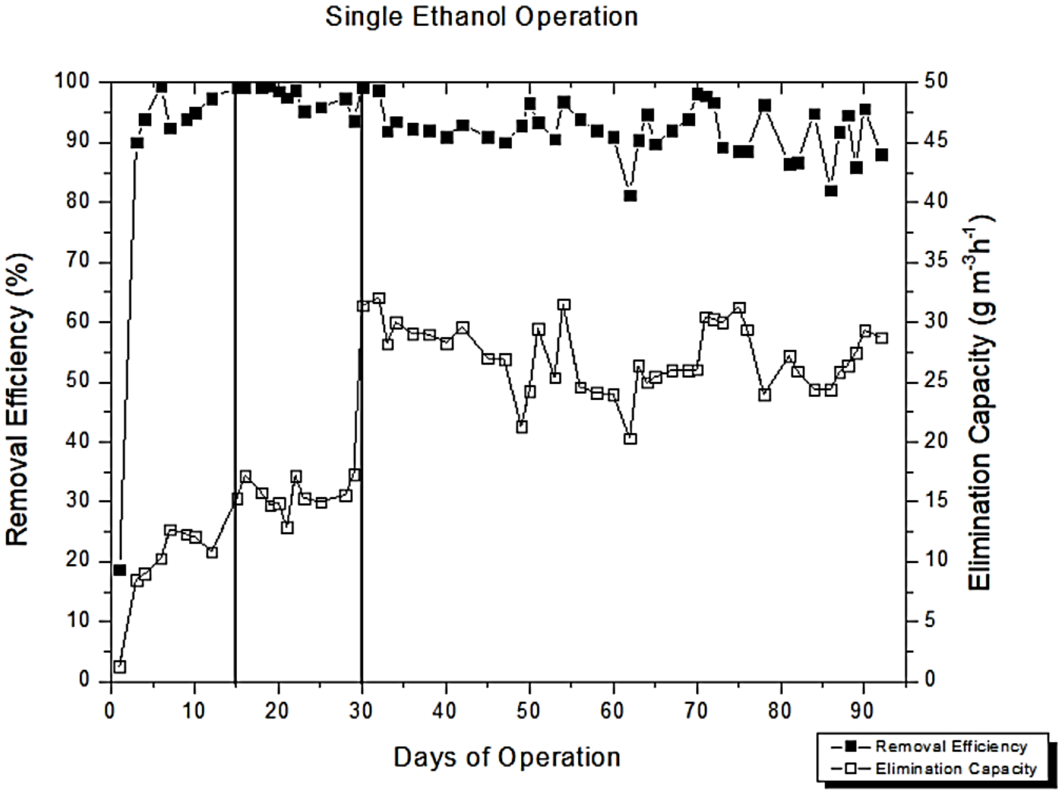

Figure 3.3 : Time course of ethanol removal efficiency and elimination capacity . Vertical lines represent different operating stages as indicated in the upper part of the figure.

During the first three stage of operation E was successfully removed when it represent elimination as a single component in the system. At the day 93, a mass transfer test was performed to understand if the system was limited either by mass transfer or by biological activity for further step experiments (mixture emissions). Hourly samples from inlet and outlet of the biofilter column were taken and the results were shown in Figure 3.4 and the effect of mass transfer to EC and RE was shown in Figure 3.5. Increase at inlet E concentration made it clear that microbial community doubled the biodegradation capacity with only slight decrease on RE. These results showed the system was not biologically limited and the enhancement by mass transfer will improve performance of the biofilter. This enhancement could increase mineralization capacity of microorganisms in the biofilter column.

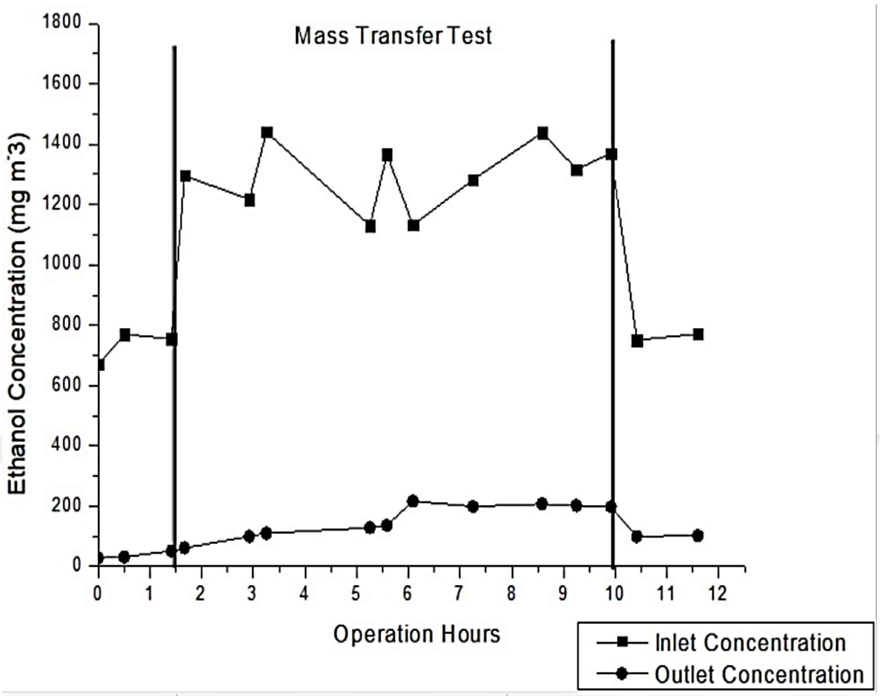

Figure 3.4 : Inlet and outlet concentrations of ethanol during the mass transfer test.

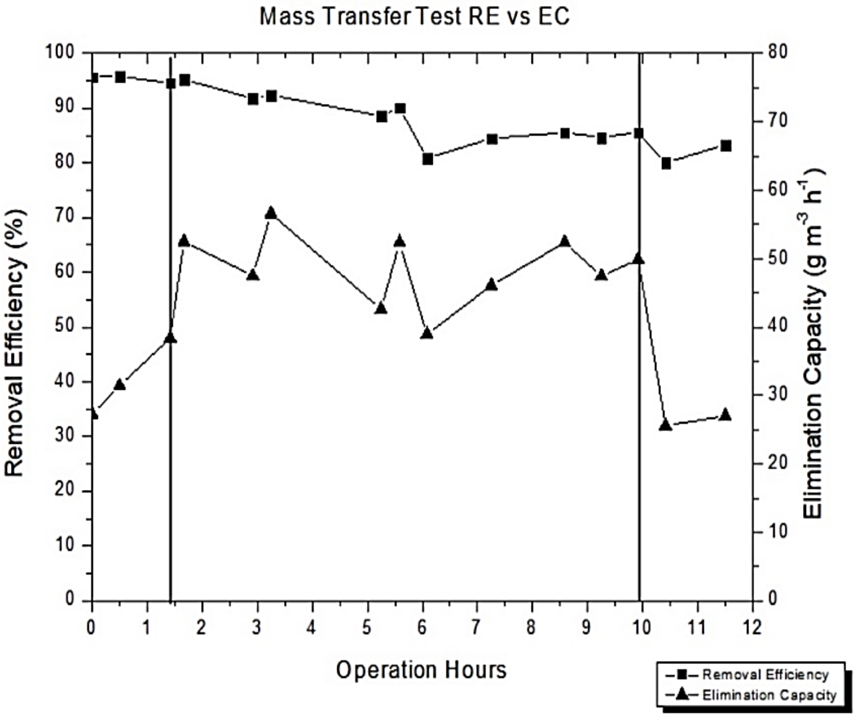

Figure 3.5 : Ethanol removal efficiency and elimination capacity during the mass transfer test.

To simulate entire waste gas stream of food fermentation process cycle, AD and A were added to synthetic gas stream together with E at the day 93th day of operation. Herewith, inlet gas stream constituted emissions; 647 ± 97 mg m^{-3} of E, 255 ± 48 mg m^{-3} of AD and 68 ± 16 mg m^{-3} for A to simulate peak process conditions in Figure 3.6**.**

At the beginning of the ternary gas mixture feeding regime, E removal was not affected from the variaty of the other compounds. AD removal pattern was recorded as similar to E and AD was removed with RE of 89±1 % and an E.C of 9.7 ±1.2 g m^{-3} h^{-1} by the day 99 while on the contrast A removal was attained only partly corresponding to R.E of 28±1 % and an E.C of 1±0.2 g m^{-3} (Figure 6). After 10 days of adaptation period to ternary waste gas stream, step by step A removal doubled itself and E.C reached to 1.9 ±0.2 g m^{-3}, while E and AC removal maintained higher than 90 %. At the day 122 flowrate of the gas stream increased 50 % and reached to 3 m^3 h^{-1} that decreased the EBRT to 53 seconds. As a result of high organic load removal of all compounds decreased system reached to minimum removal efficiency a by day 128 with R.E. of 50±1 %, 64±1%, 20±1 % for E, AD and A respectively corresponding lowest E.C. at day 128 with 14.6 ±1.2 g m^{-3}, 9±1.2 g m^{-3}, 1.1 ±0.3 g m^{-3} for E, AD and A. The detrimental effect of high organic input pointed before in many previous study due to possible antagonistic reactions during simultaneous biodegradation of multicomponent under high organic loads. That could result to possible inhibiting by-roducts production (Jiang et al., 2015; Yang et al., 2018). This could be an explanation for the detrimental effect of organic load increase on biodegradation rates. To overcome the negative effect of high organic load, system returned to previous steady state conditions by the day 129 and capable to recover itself in a short time of period and biofilter operation ended at the day 144 after reaching R.E. of 92±1 %, 86±2 % , 62±1 % with E.C. 38.5 ±1.9 g m^{-3} , 12.4±1.1 g m^{-3} , 2.5 ±0.3 g m^{-3} for E, AC and A, respectively based on the problems of air supply to the system.

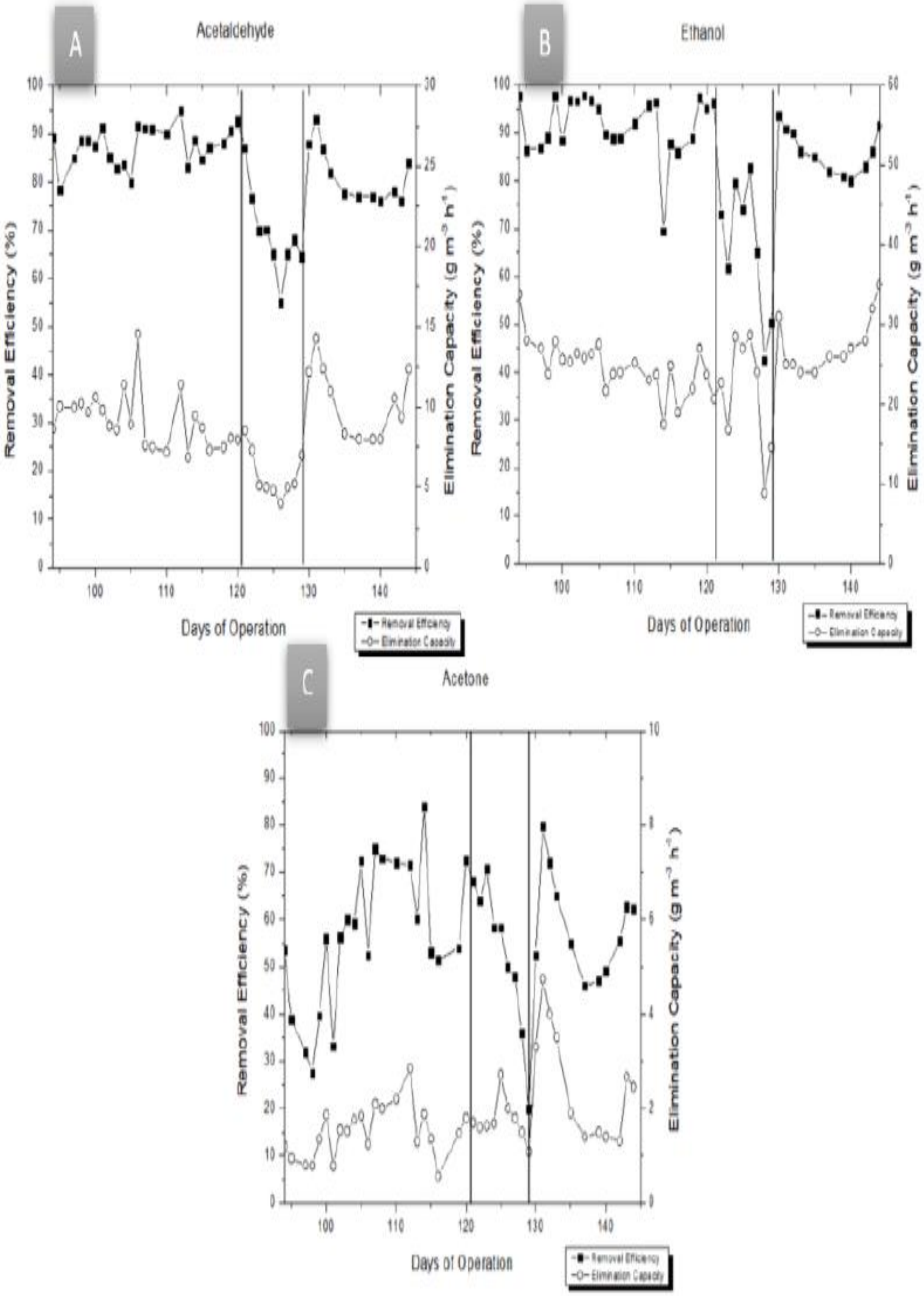

Figure 3.6 : Time course of acetaldehyde(A), ethanol(B) and acetone(C) removal efficiency and elimination capacity. Vertical lines represent different operating stages as indicated in the upper part of the figure.

4. ANOXIC DENITRIFICATION OF BTEX: BIODEGRADATION KINETICS AND POLLUTANT INTERACTIONS

With the aim of this chapter emissions such as benzene, toluene, ethylbenzene and xylene (BTEX) that frequently released to atmosphere in the second model sector of petrochemical industry were investigated to understand biodegradation pathways during their O_2-free oxidation due to the potential explosion risk of emissons in the presence of oxygen. As it mentioned the extensive use of BTEX is often criticized through their hazardous effects to the human health and on environment andits urgent need to higlight their biodegradation under O_2 free conditions. Up to date most of previous studies were highlighting the biodegradation kinetics of individual BTEX compounds under aerobic conditions but on the other hand there is a lack of literature to determine degradation kinetics of BTEX for O_2 environment. Moreover interactions of multiple carbon sources (BTEX) under the O_2 free environment are still in scar scale Therefore the kinetic parameter estimation come into prominant to evaluate the impacts of changes in variables, such as operating conditions on system performances. In this regard, estimation kinetic parameters of BTEX under anoxic conditions will help to understand biodegradation pathways of microorganisms and will overcome the problem of incorrect process design and modelling of biodegradation process

With the aim of objective this study evaluation of the biodegradation of BTEX under anoxic conditions were investigated in 1.1 Liter batch serum bottles when BTEX compounds were present as single substrates and as dual/quaternary mixtures. In addition to, the biodegradation kinetic parameter estimation for single, dual and quaternary BTEX mixtures were carried out with use of two mathematical models: Monod model and a Modified Gompertz model. Moreover, the influence of the type of BTEX compound or BTEX mixture on the structure of the microbial community was also assessed.

4.1 Inoculum

1.1 Liter air-tight serum bottles were inoculated from the the anoxic denitrification tank of the urban wastewater treatment plant (WWTP) of Valladolid (Spain). In order to have microbial characterization one of the samples of the raw sludge was was stored at 253 K.

4.2 Chemicals and Mineral Salt Medium

Chemicals for the compounds benzene, toluene, ethylbenzene and o-xylene (99.0% purity) were attained from SigmaeAldrich (Madrid, Spain). Chemicals that used for mineral salt medium (MSM) preparation were purchased from PANREAC (Barcelona, Spain) with at least 99% purity. During the batch test assays MSM solution that composed of (kg m^{-3}): $Na_2HPO_4.12H_2O$, 6.15; KH_2PO_4, 1.52; $MgSO_4.7H_2O$, 0.2; $CaCl_2$, 0.038; and 10 mL L^{-1} of a trace element solution containing (kgm^{-3}): EDTA, 0.5; $FeSO_4.7H_2O$, 0.2; $ZnSO_4.7H_2O$, 0.01; $MnCl_2.4H_2O$, 0.003; H_3BO_3, 0.03; $CoCl_2.6H_2O$, 0.02; $CuCl_2.2H_2O$, 0.001; $NiCl_2.6H_2O$, 0.002; $NaMoO_4.2H_2O$, 0.003 were prepared and used (Muńoz et al., 2013). pH of the MSM always setted to 7 before of usage. To supply electron acceptor under anoxic conditions Nitrate (NO_3^-) (supplemented as $NaNO_3$) was used for two aims as the nitrogen source for microbial growth and as well as BTEX oxidation.

4.3 Experimental Procedure

During the batch test assays all combination of BTEX mixtures were carried in air-tight bottles of 1.1 L with 100mL of MSM and they were inoculated with 5mL of the inoculum to obtain a final concentration of ~200mg L^{-1} of volatile suspended solids (VSS). In order to have controls to rule out any potential abiotic BTEX two extra serum bottles containing acidified MSM (pH = 2) to prevent any biological activity and without inoculum were used. Agitation at 320 rpm were used continusly by magnetic stirring and room temperature maintained at 298 K during the entire test. After inoculation, The headspace of the bottles was purged with N_2 (Abello Linde, Spain, purity >99.999%), with aim to remove all the oxygen present in the initial air atmosphere for at least 10 min (GC/ TCD analysis were carried out from the headspace of bottles to determine the time required to reach anoxic conditions). For all scenarious batch test assays were done in dublicate to study the degradation of these pollutants individually (4 experimental conditions), as dual mixtures (6 experimental conditions) and in the presence of the four BTEX simultaneously (1 experimental condition). For the control bottle that contained the acidifed MSM all BTEX mixture was added. Each compound (BTEX) injected to air-tight bottles in liquid form with precision syringe.The syringe was kept during 3 min after injection in the bottle headspace in order to be sure complete volatilization. Table 4.1 illustrated the BTEX concentration achieved in the headspace corresponded to the typical VOC concentrations were noticed in biological waste gas treatment (ranging between 300 and 700 mg m^{-3}) and in passive emissions from gasoline (~200 μg m^{-3}) (Barona et al., 2007; Khan et al., 2018).

4.4 Culture Enrichment Assays

In order to acclimate microorganism to BTEX waste gas stream at the beginning of the acclimation period, the corresponding BTEX were added to the headspace of the bottles to the initial concentration of each assay and illustrated in Table 4.1. Daily samples were analysed via using a gas-tight syringe (Hamilton, USA) by GC-FID. When BTEX concentration in the headspace was completely depleted, it has considered as one cycle. In some points especially for B and X and their presence in dual combination stopped the biodegradation before complete pollutant depletion based on accumulation of metabolies. At this point 30% of the cultivation broth was renewed by biomass centrifugation and resuspension in fresh MSM, with the aim of premediate the biodegradation of the remaining pollutant. After completing each biodegradation cycle, the BTEX content was readded to the batch test bottles the pertaining quantity of compounds to restore the initial concentration in Figure 4.1.

To supply nutrients for microbial growth, 30% of the cultivation broth was weekly exchanged with fresh MSM. To prevent biomass loss, cultivation broth extracted was returned to the batch test bottles by centrifugation and resuspension in the fresh MSM. As a result, during the acclimation period, the bottles operated as fed-batch at hydraulic retention time of ~23 d and 100% of biomass retention.

Lastly, NO_3^- was essential to continue the microbial activity due to being electron acceptor under anoxic conditions and required NO_3^- supplemented from a 10 g L^{-1} $NaNO_3$ stock solution. Theoretical value for complete biodegration were calculated and always twice the theoretical value required for total pollutant degradation were added to the systems to prevent NO_3^- limitation. HPLC-IC was used prior NO_3^- supplementation for nitrate analysis.

Minimum five biodegradation cycles were completed for acclimation under each experimental condition using BTEX as a single energy and carbon source before the comparative kinetic assays were performed.

4.5 Kinetic assay

Five cycle of complete biodegradation were carried out to acclimate microorganisms to BTEX components before the start-up of kinetic assay experiments. Primarly 30% of the cultivation broth was exchanged with fresh sterile MSM before the kinetic assay experiment and supplemented with $NaNO_3$ to make it sure that sufficient nutrients and nitrate were added during the test, the biomass being recovered and returned to the bottle. After successfully exchanging the medium the specific compound or mixture were added to the bottle headspace and experiments were began and shown in Table 4.1 and Figure 4.1. The BTEX concentration in the headspace was controlled in every 40 min and measurements were carried in GC/FID until complete BTEX depletion or until BTEX biodegradation stopped. At the end of the each kinetic assays, samples for VSS and NO_3^- quantification and for molecular analyses were taken. To prevent deteriotation of samples collected for molecular analyses were immediately stored at 253 K.

Table 4.1 : Initial BTEX concentration in the batch tests and duration of the acclimation period for each experimental condition (T -toluene; E-ethylbenzene; X-xylene; B-benzene).

Experiment		*Concentration (mg m^{-3})*				*Acclimation period (d)*
		Benzene	Toluene	Ethylbenzene	Xylene	
1	B	465±33				76
2	T		564±77			29
3	E			533±40		27
4	X				564±100	115
5	B-T	560±50	557±17			79
6	B-E	495±57		584±79		104
7	B-X	479±128			511±88	154
8	T-E		499±33	595±38		21
9	T-X		439±37		440±28	91
10	E-X			560±56	552±40	107
11	B-T-E-X	552±50	541±57	600±115	553±34	146
12	Control	466±21	551±10	543±12	490±30	-

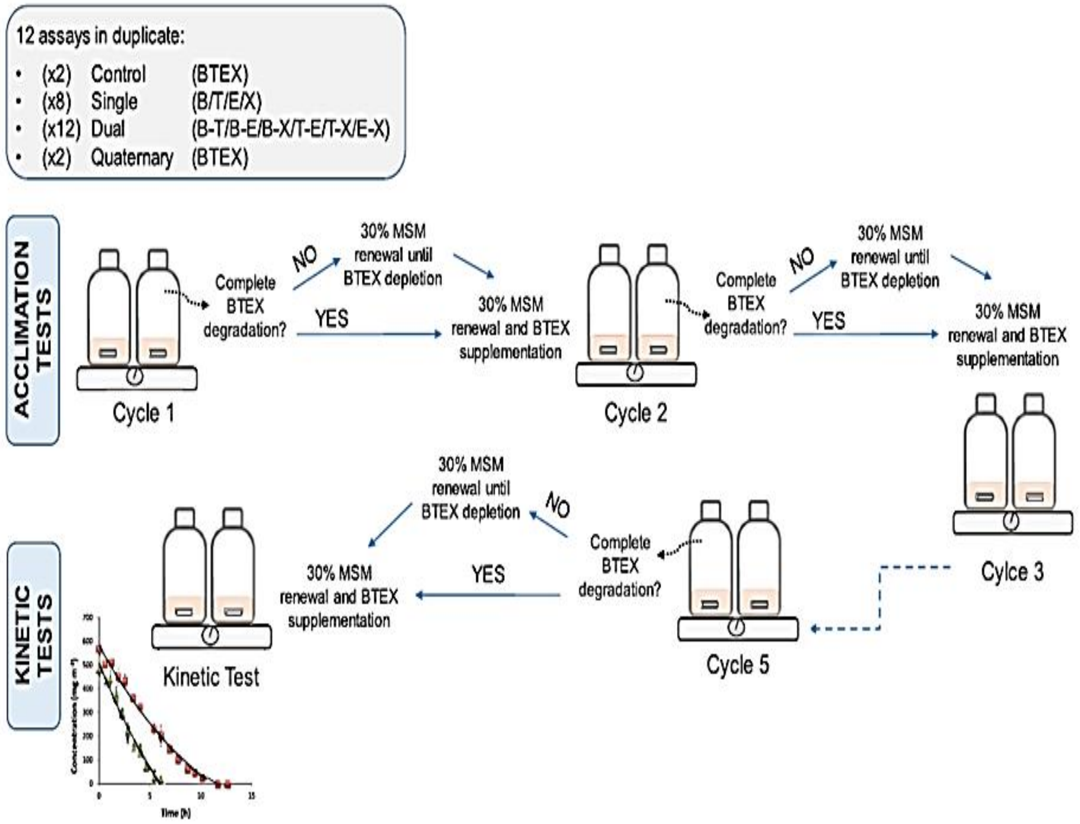

Figure 4.1 : Schematic flowchart of the experimental procedure.Analytical and microbial procedure.

Daily analyses were carried out to determine BTEX concentration in the serum bottles headspace and a Bruker 3900 gas chromatograph (Palo Alto, USA) equipped with a flame ionization detector (GC-FID) and a Supelco Wax (15m × 0.25mm × 0.25 μm) capillary column was used. Gas samples from the head space of serum bottles were taken using a gas-tight syringe (Hamilton, USA) and analysed with a method of initial temperature of oven maintained at 323 K for 60 s, increased at 0.83 K s^{-1} up to 343 K and then at 1.1 K s^{-1} to reach a final temperature of 413 K (Akmirza et al., 2017).

Standard Methods Guideline for the Examination of Water and Wastewater (AWWA, 2012) was used to determine total and volatile suspended solids (TSS and VSS concentration. Nitrite and nitrate samples were analysed in HPLC-IC using a Waters 515 pump coupled with a conductivity detector (Waters 432) and equipped with an IC-Pak Anion HC column (4.6 × 150 mm) and an IC-Pak Anion Guard-Pak (Waters). Samples were eluted isocratically at 2 mL min^{-1} (at room temperature) with a mobile phase composed of distilled water/acetonitrile/n-butanol/buffer at 84/12/2/2% v/v (the buffer solution consisted of (kg m^{-3}): $C_6H_{11}NaO_7$, 16; $Na_2B_4O_7.10H_2O$, 25; H_3BO_3, 18; and 250 mL L^{-1} of glycerol) (Muńoz et al., 2013).

Denaturing gradient gel electrophoresis (DGGE) analysis were carried out to establish the bacterial community in each BTEX combination. Samples for molecular analyses were stored at 253 K. The V6eV8 regions of the bacterial 16SrRNA genes were amplified by Polymerase Chain Reaction (PCR) using the universal bacterial primers 968-F-GC and 1401-R (SigmaeAldrich, St. Louis, MO, USA). The analysis was carried out according to Lebrero et al. (2012). The DGGE analysis of the amplicons was performed with a D-Code Universal Mutation Detection System (Bio Rad Laboratories) using 8% (w/v) polyacrylamide gels with a urea/formamide denaturing gradient of 45e65%. DGGE running conditions were applied according to Roest et al. (2005). The desired DGGE bands were excised from the DGGE gel in order to elucidate the bacterial composition of each sample. The procedure was previously described in Lebrero et al. (2012).

GelCompar IITM software (Applied Maths BVBA, Sint-Martens-Latem, Belgium) was used to process the DGGE profiles of samples. The Shannon-Wiener diversity index (H) was determined according to the expression: H¼_P[Pi ln(Pi)], where Pi is the importance probability of the bands in a lane, and is calculated as ni/n, where ni is the height of an individual peak, and n the sum of all peak heights in the densitometric curves of the DGGE profile. Thus, this index reflects both the sample richness (relative number of DGGE bands) and evenness (relative intensity of every band). Previous studies ranges it from 1.5 to 3.5 (low and high species evenness and richness, respectively). Similarity indices of the compared profiles were calculated from the densitometric curves of the scanned DGGE profiles by using the Pearson product-moment correlation coefficient (Hane et al., 1993). The taxonomic position of the sequenced DGGE bands was obtained by the RDP (Ribosomal Database Project) classifier tool at a confidence level of 50% (Wang et al., 2007). Moreover the Blast search tool at the National Centre for Biotechnology Information (McGinnis and Madden, 2004) resulted the closet match to every band. The sequences were deposited in the GenBank database under accession numbers KU991963eKU991989.

4.5.1 Kinetic modelling

Many different models have been used until now to decsribe microbial growth and pollutant biodegradation kinetics under different environmental conditions, among the mathematical models the Monod model offers a suitable approach of the batch growth mode, especially when describing the biodegradation of a single substrate in equation 4.1 (Blok, 1994; Dette et al., 2005):

$$\mu = \frac{\mu_{max} S}{K_S + S} \tag{4.1}$$

where μ stands for the specific growth rate (time $^{-1}$), μ_{max} for the maximum specific growth rate, S for the concentration of the limiting growth substrate (mg m^{-3}) and K_S represents the half-velocity constant (mg m^{-3}). In addition this monod model is commonly used to decsribe biomass growth rate also presented in equation 4.2 according to the following mass balance (X standing for the total biomass concentration):

$$\boldsymbol{\frac{dX}{dt} = \mu X} \tag{4.2}$$

Morever, substrate concentration (S) can be decsribed versus time during degradation in equation 4.3.

$$\frac{-dS}{dt} = \frac{\mu X}{Y_{XS}} \tag{4.3}$$

where Y_{xs} is the biomass yield coefficient . At this point It should be noted that Yxs presents the consumption of gas and dissolved pollutants in batch systems, where it assumes a non-limiting gas-liquid mass transfer by the reason of the high volatility of BTEX. Within this scope, the Monod model accurately estimates maximum specific growth rate and half-velocity constant for single substrate biodegradation processes where no lag phase exists. However, the existence of multiple substrates can remain the uncertainty during the modelling of substrate degradation due to possible substrate interactions. This uncertainty can be decreased within the use of a modified Monod model and that can provide a better fit to experimental data by including an inhibition constant (K_I) as in Andrews model and shown in equation 4.4.

$$\mu = \frac{\mu_{max} S}{K_S + S + S^2/K_I} \tag{4.4}$$

On the contrast, multiple non-linear regression models have been used on the previous studies to describe only microbial growth without including the consumption of the substrate (Zwietering et al., 1990; Whiting, 1995). Such as , the Modified Gompertz Kinetic model in equation 4.5 will provide the cumulative production (P) as a function of time, and may offer a better fit to experimental data in systems for multiple substrates:

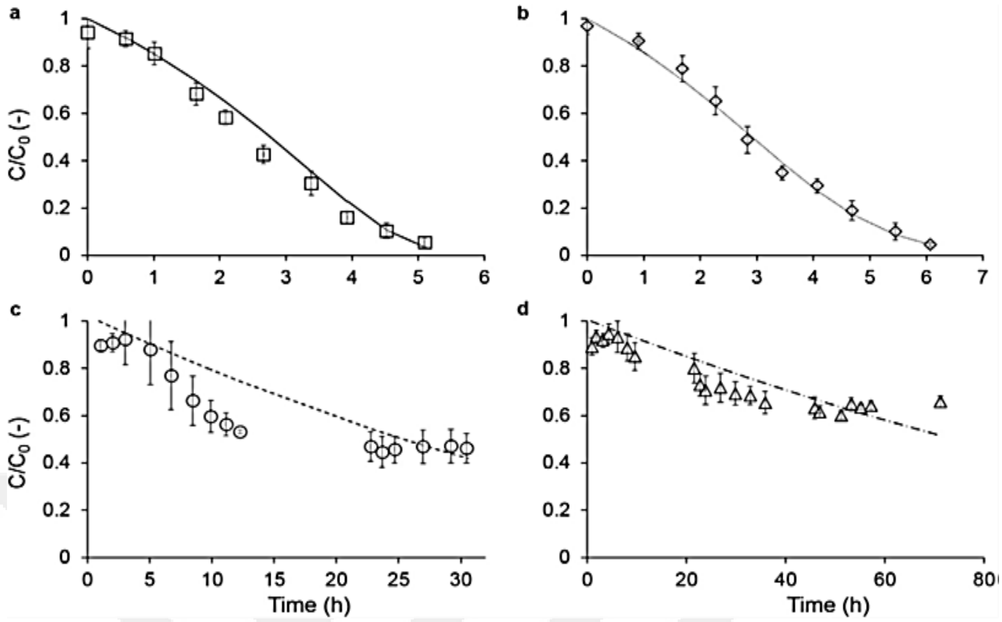

Figure 4.2 : Time course of the relative concentration (C/Co) of (a) Toluene (□.¬), (b) Ethylbenzene (◊ ◦◦◦), (c) Xylene (○ ----) and Benzene (▲¬ ◦ ¬). Symbols represent experimental data, while the lines represent the Monod model fitting.Vertical lines represent the standard deviation between duplicates of the same experimental condition.

$$P(t) = P_{max}\, exp\left(-exp\,\left(\frac{R_m}{D_{max}}(\lambda - t) + 1\right)\right) \tag{4.5}$$

where, R$_m$ represents the maximum rate of production, while P$_{max}$ is the maximum production, t is the elapsed time and λ the lag-phase, that can not be predicted with the use of Monod model. The Modified Gompertz Kinetic model offers an advantage to describe substrate consumption as a degradation potential using the equation 4.6. In our particular context, the new variables used are the cumulative degradation (D) and the maximum degradation potential (D$_{max}$).

$$\boldsymbol{D(t) = D_{max}\, exp\left(-exp\,\left(\frac{R_m}{D_{max}}(\lambda - t) + 1\right)\right)} \tag{4.6}$$

4.5.2 Data analysis

Non linear curve fitting was fullfiled for each data set to estimate the kinetic parameters of the used models. In the special occasion of Monod model (Equation (4.1)), substrate concentration versus time was fitted within the use of the numerically integrated substrate mass balance (Equation (4.3)). For the Modified Gompertz model (Equation (4.5)), the degradation (D) versus time data set was directly fitted (D (t) calculated as in equation 4.7 with C_0 being the initial concentration of the pollutant and Ci the concentration as a function of time). In both cases the initial values of the parameters were estimated visually and constrained to values higher than 0.

$$D(t) = \frac{(c_0 - c_i)}{c_0} \tag{4.7}$$

Parameter determination using the GRG Nonlinear resolution method was done within The Solver tool of the Excel Software®. The square of sum of residuals (RSS) between experimental and calculated data was minimized in the optimization process. The adequacy of the calculated parameters was confirmed by: 1) plotting residual vs. predicted data, and 2) calculating the R^2 value in equation 4.8.

$$\boldsymbol{R^2 = 1 - \frac{S^2(D_{exp})}{S^2(residuals)}} \tag{4.8}$$

Lastly, the estimated parameters using Andrews model (Equation (4)) did not improve the fitting obtained with the Monod and the Modified Gomperzt models, thus the results are not presented in this thesis work.

For statistical comparison purposes, the 95% confidence bounds of each calculated parameter were determined using the nlparci function of Matlab Software®.

4.6 Results and Discussion

4.6.1 Kinetics of individual BTEX biodegradation

After the 5th pollutant acclimation period which were differenciated (lasting 29, 27, 76 and 115 d for toluene, ethyl-benzene, benzene and xylene, respectively), kinetic parameter estimation assays were fullfilled obtaining characteristic substrate degradation profiles for individual substrates in Figure 4.2. Complete metabolization were observed for both E and T when they presented as single energy and source sources in their serum bottles. Maximum spesific growth rate were determined as 0.473 h^{-1} and 0.318 h^{-1}, respectively, and elapsed biodegradation periods of 5 and 6 h for T and E, respectively and presented in Table 4.2.

Table 4.2 : Kinetic parameters for single compound degradation tests (T - toluene; E - ethylbenzene; X - xylene; B - benzene; K_S - half velocity parameter; u_{max} - maximum specific growth rate;Y_{XS} - biomass yield coefficient; Dmax - maximum degradation potential; R_m - maximum roduction rate; λ - lag-phase).

	T	E	B	X
Monod Model				
K_s (mg m^{-3})	73	268	634	491
μ_{max} (h^{-1})	0.318	0.473	0.001	0.001
Yxs (mg g^{-1})	1.000	1.000	0.006	0.001
R^2	0.972	0.979	0.763	0.825
Gompertz Model				
D_{max} (mg mg^{-1})	1.000	1.000	0.329	0.496
R_m (mg mg^{-1})	0.812	0.697	0.035	0.187
λ - lag-phase	0.907	0.949	7.021	4.313
R^2	0.984	0.983	0.908	0.910

These results, also observed during the acclimation period, confirmed that those pollutants were the most readily biodegradable under anoxic conditions among the aromatic compounds tested. Toluene and ethyl-benzene have been alsoconfirmed as readily biodegradable under aerobic conditions when present as the sole carbon and energy source, with mmax values reported in literature within the same order of magnitude to those obtained in the present study (a summary of the μ_{max} values obtained in previous studies is presented in Table 4.2)

On the contrast to T and E, B and X were recalcitant compounds and they were not completaly metabolized by the consortium despite the long acclimation time provided; in fact, several MSM replacements were necassary for complete depletion during the acclimation period. At this case, the concentration of B could only decrease by 34 ± 9% within the first 11 h when it was used as the sole carbon and energy source (B concentration stabilizing afterwards), while X could achive only a maximum removal of 49 ± 4% within the first 24 h. The possible accumulation of toxic intermediates in the liquid cultivation media resulted to the inhibition of both X and B biodegradation where similar to the pattern observed during the acclimation cycles. This entailed low μ_{max} values and high t_∞, which accounted for 0.001 h^{-1} and 30 h for X and 0.001 h^{-1} and 70 h for B, respectively. The high recalcitrant character of both X and B has been also reported by previous studies under aerobic conditions, where X and B are typically the least favorable biodegradable BTEX compounds by indigenous microorganisms under oxygen-limited conditions (Shim and Yang, 1999; Trigueros et al., 2010). However, μ_{max} up to two orders of magnitude higher than those found in this study for B have been also observed with the presense of oxygen in both pure cultures and bacterial consortia. On the contrast, the μ_{max} values reported in the previous stuides for xylene biodegradation by pure cultures were similar or even lower, and some studies have pointed out the inability of some bacterial consortia to metabolize X when X present as the only energy and carbon source (Littlejohns and Daugulis, 2008; El-Naas et al., 2014). K_s values which were predicted with the Monod model also supported these findings. It also confirmed the higher affinity of the culture for T than for the other compounds Table 4.2. It is worth noting that the half-saturations constants observed in this study are considerably lower than those typically observed under aerobic conditions, likely due to the low BTEX concentrations prevailing in the aqueous phase during culture enrichments. Sternly the two different behaviours observed, further confirmed by the statistical comparison of the kinetic parameter confidence intervals in Table 4.3, a different accuracy of model fitting was obtained, while T and E had a good data description within the Monod model corresponding, with R^2 of ~0.97, on the contrast a poor data description were obtained for X and B as 0.825 and 0.763, respectively. That indicates Monod model was not an efficent model to describe the biodegradation kinetics of hardly biodegradable compounds of B and X. The long lag phase of B and X were correlated with the lower accuracy (B and X presented lag phases of 7.0 and 4.3 h, respectively, while on the opposite T and E exhibited a lag phase <1 h, as estimated by the Gompertz Model) (Table 4.2) and resulted an good correlation with Monod model.

Some previous studies highlighted that lag phase created a problem for monod model fitting and it should be excluded. (Littlejohns and Daugulis, 2008), while other authors fitted the model only to the post lag phase data (Strigul et al., 2009). In addition to Ellis et al. (1996) demonstrated in their work that the Monod model under anaerobic conditions when the S_0/K_S ratio is >1, provides an proper fit of the experimental data. This finding is also valid in this work, as this ratio is clearly higher for T (>3) and E (~1.5), while S_0/K_S values near 1 were observed for X and B.

As previously mentioned, the fitting obtained for the biodegradation of B and X with Andrews model did not improve the results obtained with the Monod model. This suggested that the degradation of single substrates was not inhibited by BTEX concentration over the range of initial substrate concentrations studied (~450 -600mg m^{-3}), and confirmed the hypothesis of microbial inhibition derived from a build-up of intermediates. Other inhibition models could have been studied, but the identification and quantification of the specific inhibitory compound along with the understanding of the effect of the lag phase are necessary for a correct kinetic parameter determination, and these particular issues were out of the scope of this study. Unfortunately, the information available in literature on anoxic BTEX degradation does not provide enough data about the intermediate metabolites that could be responsible for the inhibition of the process, in contrast with the information available for BTEX degradation under aerobic or anaerobic conditions. (Vogt et al., 2011; Weelink et al., 2010). Gompertz Model was used to overcome the uncertainty problem within the use of Monod model for the recilcant compounds kinetic parameter determination due to the inhibition problem. Modified Gompertz model allows describing the lag phase, thus providing an accurate fitting for all the BTEX presented in Figure 4.3.

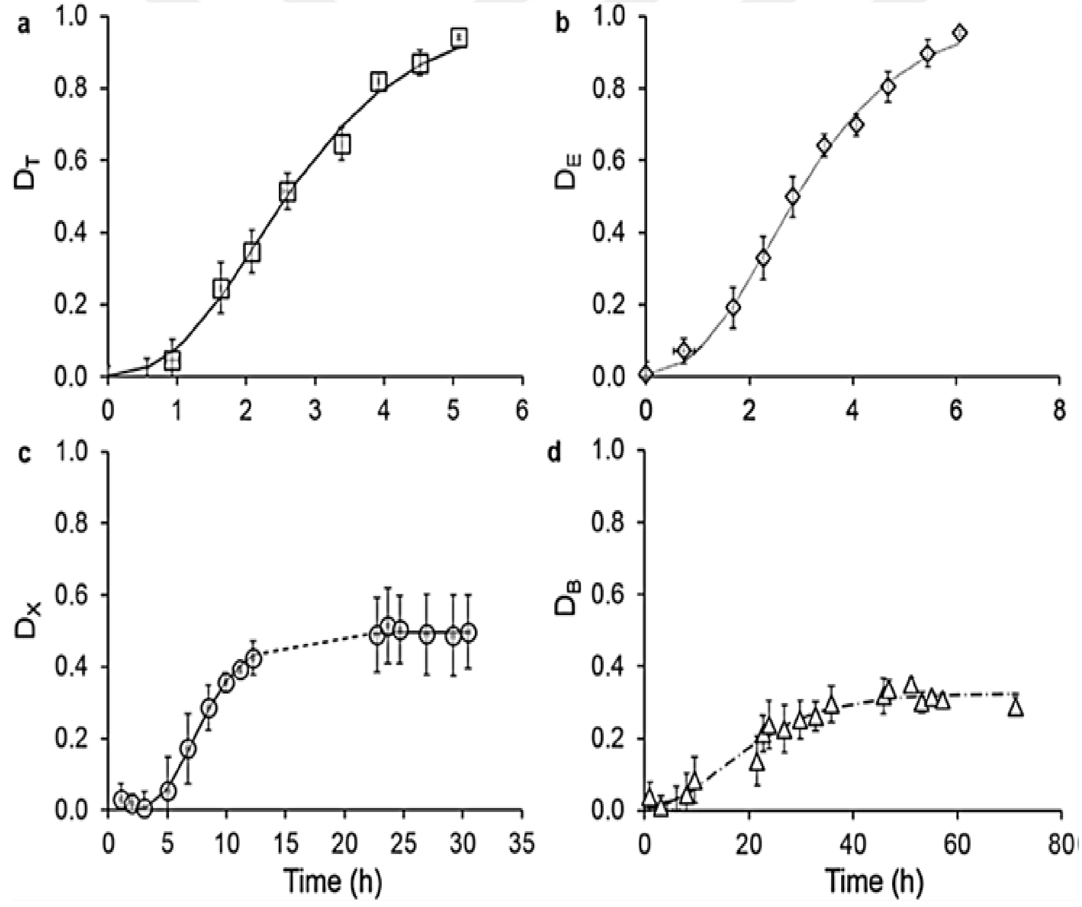

Figure 4.3 : Time course of the cumulative degradation of of (a) Toluene (□.¬), (b) Ethylbenzene (◊ ◦◦◦), (c) Xylene (○ ----) and Benzene (▲¬ ◦ ¬). Symbols represent experimental data, while the lines represent the Gompertz Model fitting. Vertical lines represent the standard deviation between duplicates of the same experimental condition.

Table 4.3 : Summary of μ_{max} values reported in previous literature studies (T - toluene; E - ethylbenzene; X - xylene; B- benzene; μ_{max} - maximum specific growth rate).

μ_{max} (h^{-1})				Inoculum Source	Model	Experimental Cond.	References
B	T	E	X				
0.3	0.33	0.27	0.24	P.putida F1	Monod	Aerobic	Trigueros et al.,2010
0.44	0.6	0.13		7 species of Pseudomonas	Monod	Aerobic	Littlejohns and Daugulis, 2008
0.135	-	-	-	Aquifer Groundwater	Monod	Aerobic	Corseuil et al.,2015
0.05	-	-	-	Aquifer Groundwater	Monod	Sulfate Reducing	Corseuil et al.,2015
0.012	-	-	-	Aquifer Groundwater	Monod	Methanogenic	Corseuil et al.,2015
0.5	0.58	-	-	P.putida F1	Monod	Aerobic	Robledo et.al,2011
0.161	0.172	-	-	P.putida F1	Monod	Aerobic	Mathur and Majumder,2010
0.16	0.26	-	0.13	-	Monod	Aerobic	Goldsmith and Balderson, 1988
0.041	0.037	0.035	0.016	P.putida F1	Monod	Aerobic	Shim et al.,2004
0.22	0.46	0.26	0.19	P.putida F1	Andrews	Aerobic	Trigueros et al.,2010
0.62	0.61	-	-	P.putida F1	Andrews	Aerobic	Abuhamed et al.,2004
0.363	0.300	-	-	P.putida F1	Linearized-Haldane’s	Aerobic	Mathur and Majumder,2010
0.019	0.006	0.034	0.006	P.aeruginosa	Haldane Model	Aerobic	Chi-Wen et al.,2007
0.41	0.42	0.45	0.05	P.putida F1	SKIP	Aerobic	Trigueros et al.,2010

Morever use of Gompertz model confirmed the complete degradation for readily biodegradable compounds of T and E (Dmax %100) with a limited lag phase (<1 h) at maximum specific degradation rates of 0.812 and 0.697mg mg^{-1} h^{-1} for T and E, respectively (Table 4.2). On the other hand, X and B exhibited lag phase values of 4.31 and 7.02 h, respectively. Xylene degradation could reach a maximum degradation 50% at a specific rate of 0.187mg mg^{-1} h^{-1} while B degradation was remained 33% by microbial commnunity with a lowest specific degradation rate apart BTEX and estimated as 0.035mg mg^{-1} h^{-1}. While the mean square regression ratios estimated for X and B were slightly lower than those calculated for T and E, the degradation kinetics of all BTEX fed as single substrates was in satisfied levels within the use of Modified Gompertz model ($R^2 > 0.9$), and it also confirmed by the statistical analysis (Table S1). To understand if the system was NO3 (electron acceptor) limited or not, liquid phase analyses were carried out and The analysis of NO3 concentration in the liquid phase confirmed that the assays were never limited by electron acceptor availability.

But, low BTEX concentrations hampered the calculation for NO3 consumption rates, resulting in negligible variations of nitrate concentration in the liquid phase.

4.6.2 Kinetics of dual BTEX mixtures biodegradation

Presense of multiple BTEX compounds in serum bottles as carbon and energy source resulted a detrimental effect over biodegradation regardless of all dual compound combination tested. The same behaviour was observed for Monod model fitting in the dual compounds biodegradation tests to that obtained for single substrates.

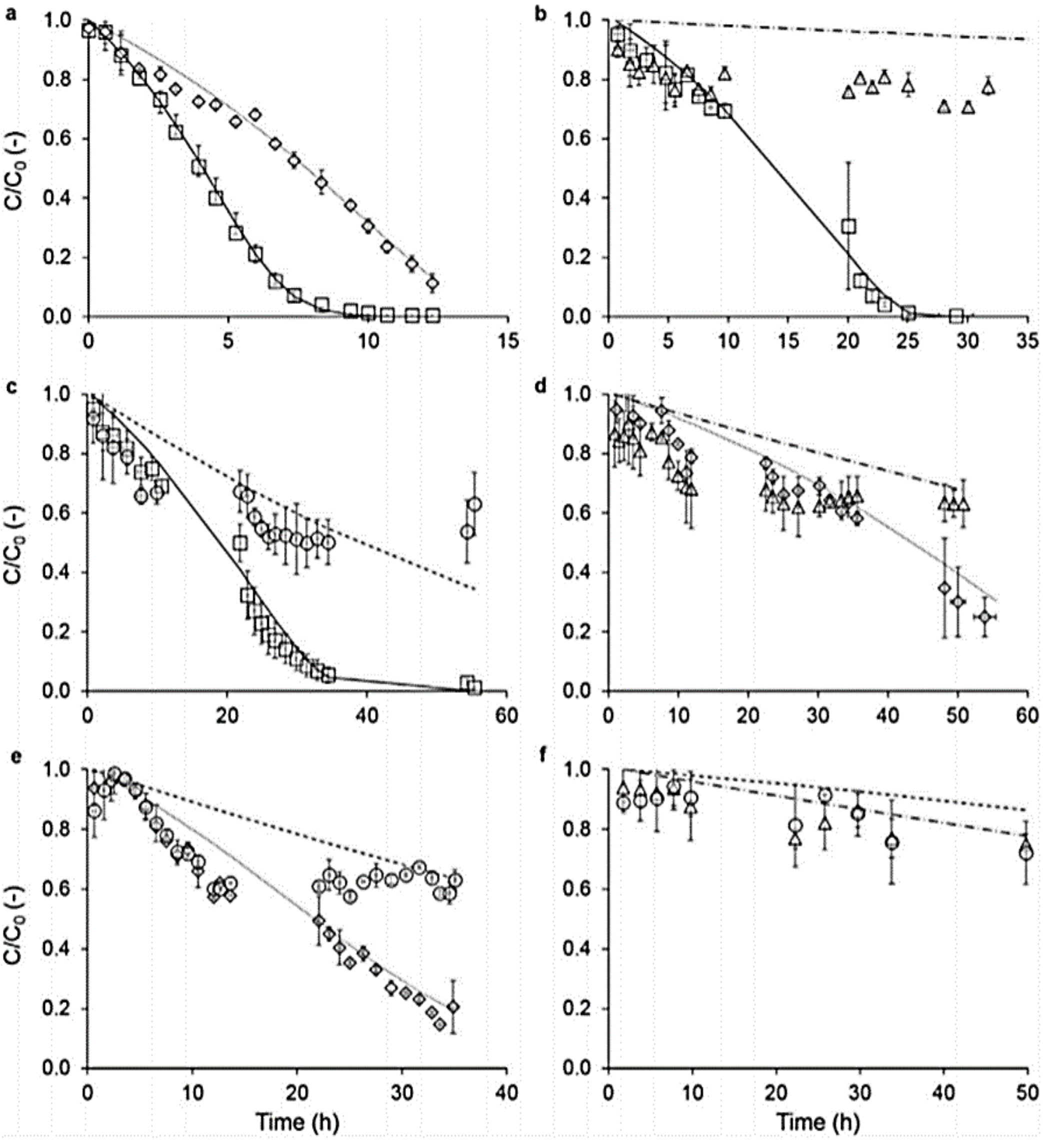

Figure 4.4 : Time course of the relative concentration (C/Co) of the dual mixtures (a) T-E, (b) T-B, (c) T-X, (d) E-B, (e) E-X, (f) X-B. Toluene (□.¬), Ethylbenzene (◊ ◦◦◦), Xylene (○ ----) and Benzene (▲¬ ◦ ¬).Symbols represent experimental data, while the lines represent the Monod model fitting. Vertical lines represent the standard deviation between duplicates of the same experimental condition.

Table 4.4 : Kinetics parameters estimated with the Monod model for dual compounds degradation tests (T - toluene; E - ethylbenzene; X - xylene; B - benzene; K_S - half velocity parameter; μ_{max} - maximum specific growth rate; Y_{XS} - biomass yield coefficient).

Mixture	Compound	K_s (mg m^{-3})	μ_{max} (h^{-1})	Yxs (mg g^{-1})	R^2
T-E	T	175	0.297	1.000	0.994
	E	56	0.086	0.570	0.987
T-B	T	61	1.328	0.624	0.975
	B	515	0.001	0.004	0.770
T-X	T	79	0.037	0.563	0.977
	X	622	0.001	0.010	0.407
E-B	E	46	0.020	1.000	0.908
	B	451	0.001	0.030	0.578
E-X	E	459	0.079	0.915	0.957
	X	450	0.001	0.018	0.417
B-X	B	436	0.001	0.045	0.682
	X	62	0.008	1.000	0.550

Table 4.5 : Kinetics parameters estimated with Gompertz model for dual compounds degradation tests (T - toluene; E - ethylbenzene; X - xylene; B - benzene; D_{max} - maximum degradation potential; R_m - maximum production rate; λ - lag-phase).

Mixture	*Compound*	D_{max} *(mg mg^{-1})*	R_m*(mg $g^{-1}h^{-1}$)*	λ - lag-phase	R^2
T-E	T	1.000	0.518	1.428	0.989
	E	1.000	0.220	1.403	0.957
T-B	T	1.000	0.139	2.287	0.936
	B	0.192	0.088	0.385	0.650
T-X	T	1.000	0.102	2.602	0.976
	X	0.411	0.090	0.353	0.855
E-B	E	1.000	0.034	3.704	0.857
	B	0.262	0.127	6.403	0.696
E-X	E	0.921	0.079	2.257	0.856
	X	0.336	0.170	4.598	0.927
B-X	B	0.156	0.367	9.382	0.570
	X	0.189	0.442	32.761	0.522

All T and E combinations showed a proper fitting, while lower R^2 values than those obtained during individual BTEX biodegradation were achieved for mixtures containing B and/or X (Figure 4.4, Table 4.4). Instead of that Modified Gompertz model could describe the dual BTEX mixtures biodegradation assays with high suitability in Figure 4.5, except B biodegradation, which had a R^2 lower than 0.7 regardless of the mixture illustrated in Table 4.5. It can be explained within small scarce This result could be explained in small quantities of contaminant degradition which remained always lower than 26%.

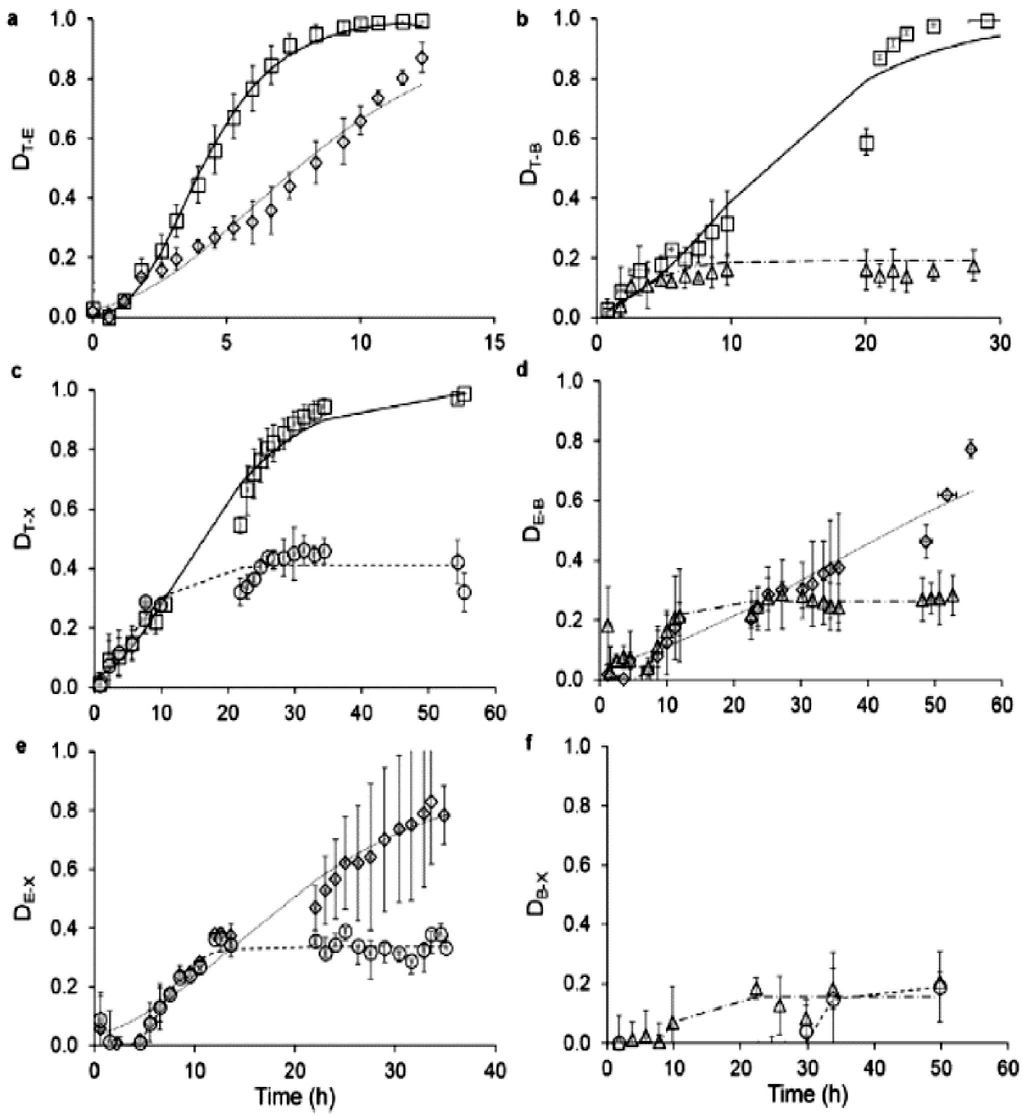

Figure 4.5 : Time course of the cumulative degradation of (a) T-E, (b) T-B, (c) T-X, (d) E-B, (e) E-X, (f) X-B. Symbols represent experimental data, while the lines represent the Gompertz Model fitting. Vertical lines represent the standard deviation between duplicates of the same experimental condition.

On the contrast, Andrews model failed to proper estimation of the kinetic parameters of the dual BTEX mixtures degradation. Actually, the values obtained for the inhibition parameter (K_I) were very high for the data sets that previously fitted the Monod model, reaching the maximum value allowed by the optimization (fixed as bound). On the opposite, the K_I parameters for data sets that provided poor fittings were very low and again similar to the limit fixed as bound. However, no possibility for the improvement the fitting of the curves either by increasing the R^2, reducing the residues or by visual inspection. At this point of concept, previous works with the presense of O_2 have also observed that conventional mathematical models accounting for uncompetitive, non-competitive and competitive inhibition between dual BTEX mixtures do not supply an accurate fit to the experimental data (Littlejohns and Daugulis, 2008).

Presence of multiple carbon source decrased the biodegradation rates while on the opposite increased the time for complete biodegradation process comparing to indiviual substrate supplementation. For example, lag phase values were slightly higher than indiviual supplementation and ~1.4 h were recorded for T and E, and ~12 h were required for their complete degradation. Likewise, specific biodegradation rates of 0.518 and 0.220 mg mg-1 h-1 were estimated for T and E, respectively, when biodegraded together (Table 4). T was found very sensitive compound and effected from the presence of B and decelerate T biodegradation rate by a factor of 4 (down to 0.139mg mg-1 h-1), while B had a Rm of 0.088 mg mg-1 h-1 and only a partial degradation of 19% . T degradation also effected negatively from X addition and that resulted to lower specific degradation rates compared to their individual degradation (0.102 mg mg-1 h-1 and 0.090 mg mg-1 h-1 for T and X, respectively). In addition to, X degradation didnt effected from T supplementation and apart the other combinations the highest X degradation was recorded in the presence of T, with a X degradation potential of 41% and T depletion. Stumping, approximately 0.4 h were required in these tests for the start-up of B and X degradation, which were firstly metabolized by the consortia in the presence of T, while T exhibited a lag phase >2 h. The diauxic effect that also studied in previous studies for BTEX, resulting in lag phases before the preferential substrate is consumed (El-Naas et al., 2014). However the lower degradation rates were lowered for T in the dual batch biodegradation assays due to antagonistic substrate interactions, a complete T degradation was always achieved regardless of the mixture.

Both X and B resulted an negative impact on the biodegradation of E. Although, presense of B didnt have inhibitory effect over complete E biodegradation (specific biodegradation rate of 0.034 mg mg^{-1} h^{-1} with a lag phase of 3.70 h), on the contrast presense of X as co substrate resulted only 92% of E bidegradation at a specific degradation rate 0.079 mg mg^{-1} h^{-1} with ~2.3 h of lag phase. A partial degradation was recorded for B (26%) and X (34%) in dual substrate experiments supplemented with E. Lastly, in combination with B and X at the same serum bottles resulted in a negligible biodegradation of both pollutants, with only 15 and 18% of B and X degraded at specific rates of 0.367 and 0.442 mg mg-1 h-1, respectively. Moreover, these two hardly biodegradable compound combination assay resulted the highest lag phases (32.8 h for X and 9.3 h for B) . Previous works under both aerobic and anaerobic (Dou et al., 2008) conditions revealed a range of possible substrate interaction patterns including inhibition, non competative inhibition, no interaction, stimulation, (Lin et al., 2007), and cometabolism as a result of the concomitant presence of multiple BTEX compounds (Deeb and Alvarez-Cohen, 1999; Littlejohns and Daugulis, 2008).

However, these study made it clear that regardless to the type of combination with multiple carbon and energy sources resulted in an inhibitory effect over these aromatic compounds biodegradation under anoxic conditionse, with xylene inducing the highest degree of inhibition among all BTEX. It is also important to highlight that a Modified Gompertz model which is noncompetitive inhibition model, fitted accurately the experimental data for dual substrates experiments, that also suggests that microbial inhibition is caused by the inhibitor binding to the enzyme at a site other than the enzyme's active site, as also mentioned before by Lin et al. (2007). These authors reported that this type of inhibition typically results in a change in the enzyme's active site structure and ultimately in reduced pollutant biodegradation rates, as here observed.

4.6.3 Kinetics for the quaternary BTEX mixture

Combination with quarternary BTEX mixture resulted to the highest lag phase and the lowest specific degradation rates for all the compounds (Figure 4.6, Table 4.6 and S4). Highest lag phases were recorded as ~18 h for E and T, and more than 4 lower specific biodegradation rates were observed in comparison with single compound assays (0.179 and 0.151 mg mg^{-1} h^{-1} , respectively). In addition to despite E and T reached a high goodness of fit (R^2 of 0.967 and 0.987, respectively), a poor fitting was obtained when applying the Modified Gompertz model to both B and X (R^2 as low as 0.154 and 0.468, respectively). The Monod model also didnt have success to present the biodegradation of these recalcitrant pollutants, as previously observed in the single and dual experiments.

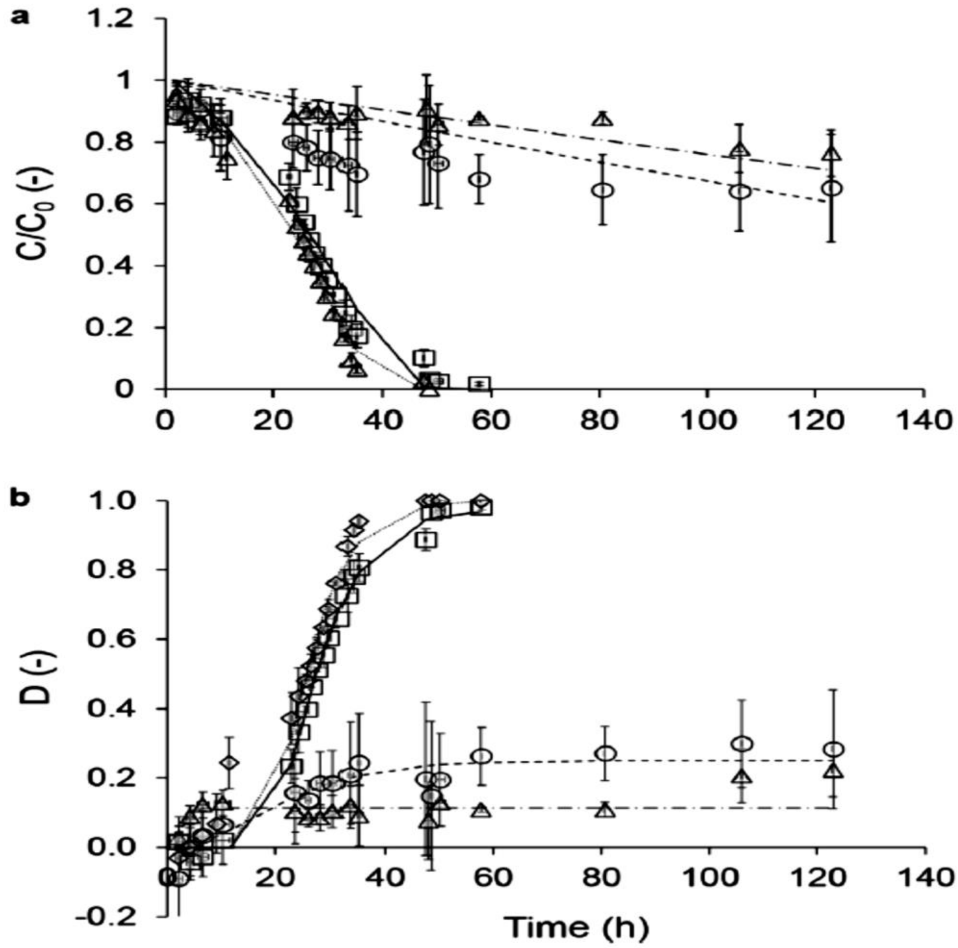

Figure 4.6 : Time course of (a) the relative concentration (C/Co) and (b) the cumulative degradation of (a) Toluene (□.¬), (b) Ethylbenzene (◊ ◦◦◦), (c) Xylene (○ ----) and Benzene (▲¬ ◦ ¬) in the quaternary BTEX mixture. Symbols represent experimental data, while the lines represent the Monod Model (5a) and the Gompertz Model (5b) fitting. Vertical lines represent the standard deviation between duplicates of the same experimental condition.

Table 4.6 : Kinetic parameters of the quaternary BTEX mixture degradation test (T - toluene; E - ethylbenzene; X - xylene; B - benzene; KS - half velocity parameter; μ_{max} - maximum specific growth rate; Y_{XS} - biomass yield coefficient; Dmax - maximum degradation potential; R_m - maximum production rate; λ - lag-phase).

	T	**E**	**B**	**X**
Monod Model				
K_s (mg m^{-3})	100	100	500	500
μ_{max} (h^{-1})	0.051	0.059	0.001	0.010
Yxs (mg g^{-1})	1.500	1.500	0.075	0.664
R^2	0.970	0.983	0.286	0.737
Gompertz Model				
D_{max} (mg mg^{-1})	0.973	1.000	0.114	0.250
R_m (mg mg^{-1})	0.151	0.179	0.122	0.022
λ - lag-phase	18.490	18.120	1.608	5.718
R^2	0.987	0.967	0.154	0.468

4.6.4 Molecular analyses

Molecular analyses were carried out to determine the microbial composition during the experimental setup. According to the results The Shannon-Wiener diversity indexes obtained from the samples studied were have great differences depending on the target compound or mixtures. The data supports that the individual BTEX biodegradation showed a higher diversity for most recalcitrant compounds, the maximum alue being recorded for xylene (HB = 3.27 and HX = 3.40). On the contrary, the indexes calculated for the community growing on the readily biodegradable compounds were lower than expected, T resultes to the the lowest diversity with (HE = 2.88 and HT = 2.10).When it comes to multiple carbon and energy sources, the diversity indexes obtained for communities treating the dual mixtures ranged from 2.50 for the mixture B-X to 3.29 for the mixture B-E. This refers that presence of recalcitrant compound in a mixture decreaed the diversity and reesulted low diversity indexes while on the contrary the individual degradation of B and X mediated a higher diversity in the microbial population. Lastly, the microbial community growing on the quaternary mixture of BTEX had low index (2.76), probably due to antagonistic BTEX interactions in the assay. Nevertheless, a relatively high richness was observed in all the samples regardless to the type of combination. This also confirmed by previous studies where it was reported that low pollutants concentrations mediate a high biodiversity, which is significantly reduced at higher pollutant concentrations (Estrada et al., 2012; Lebrero et al., 2012).

The community that was enriched in the B-X mixture had the lowest similarity with the rest of the samples acccording to the Pearson's similarity indexes where for instance, only 29% similarity was obtained with the inoculum and apart from that only 26 and 36% of similarity was recorded with the B and X communities, respectively. On the contrast, the communities enriched in either T or E remained similar and according to the index they showed similarities over 90% among them. As a result of these analyses its clear that readily biodegradable compounds did not require any specialization and their microbial diversity remained low on the contrast, the degradation of the mixture B-X also resulted in low diversity values, as above mentioned, while the degradation was rapidly inhibited probably due to metabolites accumulation (with only ~10% of the pollutants degraded). In this regard, the results were attributed to the selection of a specific group of microorganisms during the acclimation period able to grow on these recalcitrant substrates, which is also in accordance with the low similarity values observed.

From the 27 bands sequenced from the DGGE gel (Figure 4.7), 14 bands were identified as Proteobacteria, becoming the dominant phylum of this study. In addition, 5 other phyla were identified: Actinobacteria (4 band), Bacteroidetes (2 bands), Firmicutes (1 band), Deinococcus-thermus (1 band) and Verrucomicrobia (1 band). Finally, 3 bands remained as unclassified bacteria.

Species from the Alphaproteobacteria class, retrieved in all samples except in the inoculum, have been described as capable of degrading toluene with nitrate as electron acceptor (Shinoda et al., 2005). Bands 1, 2 and 3 belonged to the Rhodocyclaceae family and Thauera genus, facultative anaerobes, which have been classified as capable of performing heterotrophic and autotrophic denitrification processes, as well as able to degrade petroleum hydrocarbons in hypersaline environments (Evans et al., 1991; Xu et al., 2015). Morever, the genus Schlegelella and the Xanthomonadaceae family recorded in 2 bands have been associated to denitrification processes (bands 6 and 12), while the genus Zoogloea (bands 4 and 5) has been associated to benzene and BTEX degradation (Weelink et al., 2010; Xu et al., 2015). Lastly, the bands classified as Alphaproteobacteria (7, 8 and 9) were also found in a denitrifying process of quinoline removal (Liu et al., 2006). On the other hand, the bands identified into Actinobacteria phylum (15 and 16) have been described in several studies as VOC degraders (Lebrero et al., 2014; Akmirza et al., 2017). Moreover, Actinobacteria and Deinococcus- thermus have been associated to the biodegradation of aliphatic hydrocarbons (Militon et al., 2010). Similarly, the bands identified within the Ignavibacteriae and Firmicutes phyla (bands 23 and 25) were previously identified in samples subjected to anaerobic benzene degradation and anaerobic biodegradation of total petroleum hydrocarbons, respectively (Van der Zaan et al., 2012; Zhang and Lo, 2015).

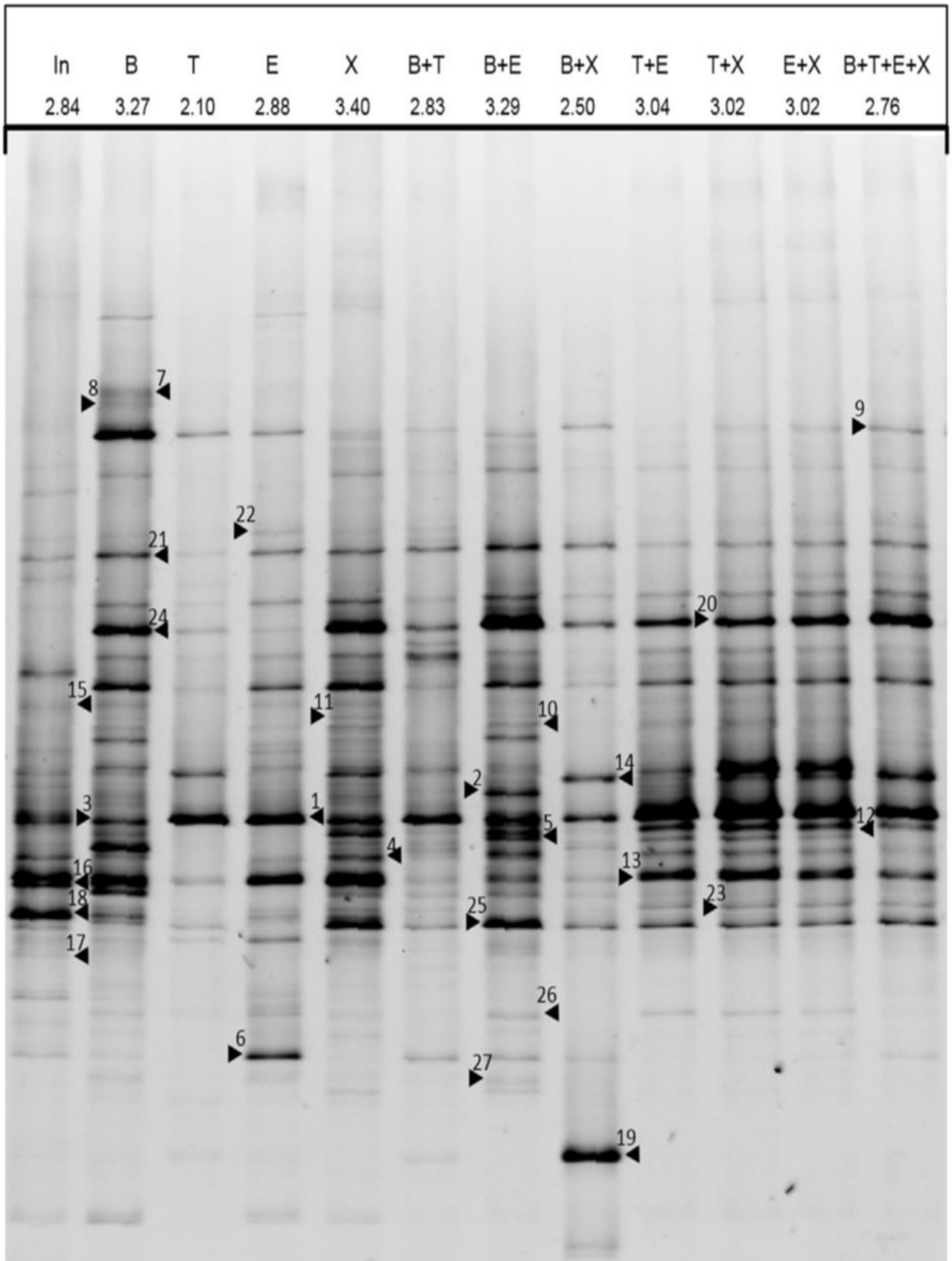

Figure 4.7 : DGGE profile of the main bacterial communities (In - Inoculum; T - toluene; E - ethylbenzene; X - xylene; B - benzene).

5. ANOXIC BIODEGRADATION OF BTEX IN A BIOTRICKLING FILTER

In this sectiom of the thesis work petrochemical industry emissions were biodegraded in a lab scale anoxic biotrickling filter for different operation conditions. Graphical abstract for the work has been presented in Figure 5.1.

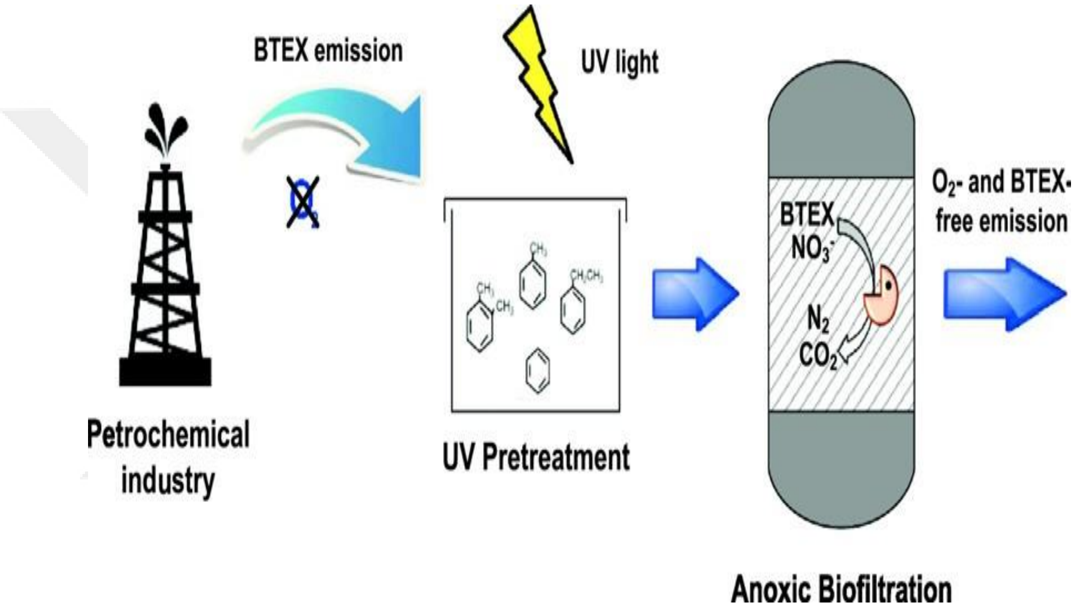

Figure 5.1 : Graphical abstract for anoxic biotrickling filter operation.

Petrochemical industry emits many volatile aromatic hydrocarbons such as benzene, toluene, ethylbenzene and xylene (BTEX) which are responsible for many important health problems such as irritation, headaches, or even liver and kidney damage and cancer (benzene has been classified as Group A-known human carcinogen by EPA) (Gallastegui et al., 2011; Wang et al., 2013). Thus, make the minimization and abatement of BTEX emissions one of the greatest importance in the sector.

Up to date, several physical-chemical treatment have been used for the abatement of BTEX emissions (Chen et al., 2010). However, their non environmental friendly nature and high capital and operation requirement have boosted the development of new biological treatment alternatives.At this point of concept, the biotreatment of BTEX provides low-cost and environmental friendly treatment alternative to physical-chemical technologies especially for high flow rated waste gas streams that is one of the most common characteristic of model sector emissions. In particular, BTFs offers multiple advantages over other biotreatment alternatives such as lower pressure drop across the packed bed due to the high porosity of the inert packing material easier control of the operating parameter (Moussavi and Mohseni, 2007; Yan et al., 2016). But still limited information is still available in literature and both positive and detrimental effects of this hybrid technology should be investigated.

At this concept of point, within this study, the potential of anoxic biofiltration for the removal of BTEX from an O2-free waste-gas stream was investigated in a laboratory scale biotrickling filter. Moreover, apart from the process parameter change, UV radiation was used as a pretreatment technology and UV was tested both in the liquid and in the gas phase before biotrickling filtration. Finally, the dynamics of the bacterial population were also assessed by DGGE-sequencing.

5.1 Materials and Methods

5.1.1 Inoculum

Activated sludge, that was also previously used for the kinetic parameter estimation of BTEX, was taken from the denitrification-nitrification wastewater treatment plant of Valladolid (Spain) and was considered as inoculum in order to shorten the acclimation time of the microbial community to anoxic pollutant biodegradation. For BTF operation, 2 L of the activated sludge were centrifuged for 10 min at 10000 rpm and resuspended in 200 mL of fresh mineral salt medium (MSM) to a final volatile suspended solids (VSS) concentration of 7580 mg L^{-1}. After the procedure inoculum was added to the biotrickling filter column which was fullfilled with rashing rings packed material and recycled through the packed bed for 1 day to promote micro- bial attachment and biofilm formation.

5.1.2 Chemicals and mineral salt medium for BTF operation

During the BTF operation chemical for MSM preparation were purchased from PANREAC (Barcelona, Spain) with a purity of at least 99%. The polluted waste gas stream, that consisted Benzene, toluene, ethyl- benzene and o-xylene (99.0% purity), were obtained from Sigma-Aldrich (Madrid, Spain). The MSM which was recirculated continously to the biotrickling filter column, was composed of (g·L−1): $Na_2HPO_4 \cdot 12H_2O$, 6.15; KH_2PO_4, 1.52; $MgSO_4 \cdot 7H_2O$, 0.2; $CaCl_2$, 0.038; and 10 mL

L^{-1} of a trace element solution containing (g L^{-1}): EDTA, 0.5; $FeSO_4{\cdot}7H_2O$, 0.2; $ZnSO_4{\cdot}7H_2O$, 0.01; $MnCl_2{\cdot}4H_2O$, 0.003; H_3BO_3, 0.03; $CoCl_2{\cdot}6H_2O$, 0.02; $CuCl_2{\cdot}2H_2O$, 0.001; $NiCl_2{\cdot}6H_2O$, 0.002; $NaMoO_4{\cdot}2H_2O$, 0.003 (Muñoz et al., 2013). NO_3^- (supplemented as $NaNO_3$ in a 8 g L^{-1} stock solution (pH, nutrient feed). BTF offers capability to treat by-products from VOCs degradation, lower area requirements with less dead zones, and higher removal efficiencies (REs) for aromatics, chlorinated hydrocarbons and organic sulfur compounds (Mudliar et al., 2010; Torretta et al., 2015).

Despite the advantages of this biotechnology, there is a limited number of studies assessing the performance of BTFs for the removal of BTEX. Besides,most of these works were conducted under aerobic conditions, limiting the application of BTFs as end-of-pipe technologies for the control of BTEX emissions from the petrochemical industry, which are present in an inert atmosphere. Therefore, the development of BTFs able to efficiently treat O_2-free BTEX emissions under anoxic conditions (using NO_3^- as electron acceptor for organic pollutant biodegradation instead of oxygen) will significantly improve the economic and environmental sustainability of VOC treatment in the petrochemical industry (Muñoz et al., 2013).

An additional limitation when removing BTEX emissions in biological reactors, together with their recalcitrant nature, is the formation of toxic by-products during the biodegradation process, which may result in process possible inhibition and thus in a significant drop in pollutant abatement performance (El-Naas et al., 2014; Moussavi and Mohseni, 2007). UV radiation can be usefull for compounds oxidation, but, solo input of technology and also its application is limited since additional by-products might be formed and emitted to the atmosphere. Thus, the combination of this physical pretreatment with a biological treatment will likely promote the breakdown of the recalcitrant BTEX and of inhibitory by-products, minimizing the toxic effects of BTEX derived metabolites and increasing system performance, since most of the by-products resulting from UV oxidation are more water soluble and biodegradable than parent compounds. In addition to, this two-stage system might also decrease the cost of operation compared with stand-alone physical technologies was used as electron acceptor for BTEX oxidation and as nitrogen source for microbial growth.

5.1.3 Experimental setup and operating procedure

Biotrickling filter at laboratory scale consisted of a cylindrical jacketed PVC column (0.084 m inner diameter, 45 cm height) packed with Kaldnes rings to a working volume of 2 L (Figure 5.2).

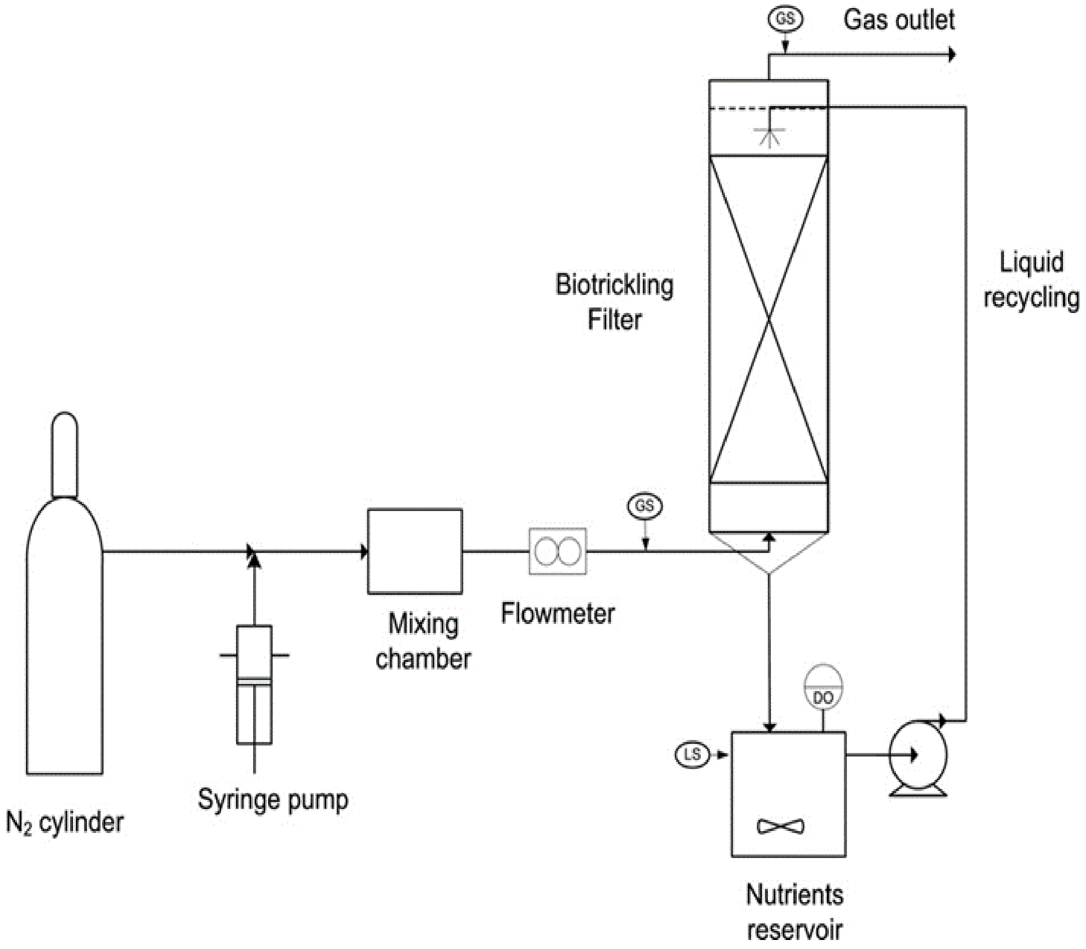

Figure 5.2 : Schematic representation of the experimental setup.

An external 1.2 L jacketed holding tank stirred at 400 rpm (Agimatic-S, Selecta®, Spain) was used as a MSM reservoir. The MSM was continuously recycled from the top of the column by a positive displacement pump (Milton Roy Iberica, G Series, Spain) at 2 m h^{-1}. The synthetic BTEX inlet stream was prepared by injecting a liquid mixture containing the four components with a syringe pump (Fusion 100, Chemyx Inc. USA) to a N2 gas stream (Abello Linde Spain, purity N 99.999%). The polluted gas stream, with a concentration of 742 ± 95 mg benzene m^{-3}, 712 ± 84 mg toluene m^{-3}, 702 ± 96 mg ethylbenzene m^{-3} and 712 ± 84 mg xylene m^{-3}, was entered from the bottom of the column in a counter current mode with the trickling liquid flow. The flow rate was controlled to maintain a gas empty bed residence time of 30 min, resulting in inlet loads of 1.4 ± 0.2 g m^{-3} h^{-1} for toluene, ethylbenzene and xylene and 1.5 ± 0.2 g m^{-3} h^{-1} for benzene.

The system was operated for 208 days (Table 5.1). From day 0 to 34, 200 mL of MSM were daily exchanged with the fresh MSM. The MSM renewal rate was subsequently increased three times to 600 mL (days 34–208). Between days 85 and 90, the pH was manually controlled at ~ 7 by addition of a 6 M HCl solution to the recycling MSM, terminating afterwards any pH control strategy due to the rapid process performance deterioration observed and in order to avoid any irreversible damage to the microbial community. Once the previous BTEX degradation performance was recovered by day 122, a 600-mL quartz photoreactor containing a UV lamp (Pen-Ray WL254 NM) was installed in the recycling liquid stream interconnecting the 1.2-L external tank with the packed bed column. The UV lamp provided an irradiation at the inner photoreactor wall of 4.0 mW cm^{-2}. Following performance stabilization, the UV photolysis pretreatment was started by day 139. By day 172, the 600 mL-quartz photo reactor was installed in the inlet gas line, the polluted stream flowing through the tank before entering the packed column. Finally, the UV light was removed by day 191 returning to the initial configuration, and the system was operated without pretreatment until day 208. Inlet and outlet BTEX and CO2 concentrations in the gas phase were daily measured using a gas-tight syringe (Hamilton, USA) by GC-FID and GC-TCD, respectively. Liquid samples were also daily drawn to de- termine the pH and the concentrations of total organic carbon (TOC), inorganic carbon (IC) and total nitrogen (TN), while nitrate (NO_3^-) and nitrite (NO_2^-) concentrations in the liquid phase were measured by HPLC-IC. Samples from the inoculum and the biofilm at the end of the operating period were drawn and frozen at −80 °C for microbial population analyses.

5.1.4 Analytical procedures

BTEX concentration in the gas phase was analysed in a Bruker 3900 gas chromatograph (Palo Alto, USA) equipped with a flame ionization detector and a Supelco Wax (15 m × 0.25 mm × 0.25 μm) capillary column.

Table 5.1 : Experimental conditions established during the eight operational stages.

Stage	Days	Dilution rate (d^{-1})	pH control	UV pretreatment	Total liquid reservoir (L)
I	0–34	0.17	No	No	1.2
II	34–85	0.5	No	No	1.2
III	85–90		Yes	No	1.2
IV	90–122		No	No	1.2
V	122–139		No	No	1.8
VI	139–172		No	Liquid line	1.8
VII	172–191		No	Gas line	1.8
VIII	191–208		No	No	1.2

Oven temperature was initially maintained at 50 °C for 1 min, increased at 50 °C min^{-1} up to 70 °C and then at 65 °C min^{-1} to a final temperature of 140 °C. CO2 concentration in the gas phase was de- termined in a Bruker 430 gas chromatograph (Palo Alto, USA) coupled with a thermal conductivity detector and equipped with a CP-Molsieve 5A (15 m × 0.53 m × 15 m) and a P-PoraBOND Q (25 m × 0.53 m × 10 m) columns. The oven, injector and detector tem- peratures were maintained at 40 °C, 150 °C and 175 °C, respectively. Helium was used as the carrier gas at 13.7 mL min^{-1}. Nitrite and nitrate concentrations in the liquid phase were analysed via HPLC-IC using a Waters 515 HPLC pump coupled with a conductivity detector (Waters 432) and equipped with an IC-PAK Anion HC column (4.6 × 150 mm) and an IC-Pak Anion Guard-Pak (Waters). Samples were eluted isocratically at 2 mL min^{-1} (at room temperature) with a solution of distilled water/acetonitrile/n-butanol/buffer at 84/12/2/2% v/v (Muñoz et al., 2013). Samples for the determination of the concentration of TOC, IC and TN in the liquid phase were measured using a Shimadzu TOC-VCSH analyzer (Japan) coupled with a TNM-1 chemiluminescence module. Biomass concentration was estimated as VSS according to Stan- dard Methods (American Water Works Association, 2012). More specif- ically, a 50 mL sample was filtered in a pre-dried and pre-weighted filter (1 µm pore size) and evaporated for 24 h at 105 °C. The corresponding residue was weighted and further dried in a furnace at 550 °C for another 24 h. The amount of VSS was determined from the resulting solid after combustion.

5.1.5 Microbiological procedure

Genomic DNA was extracted using the protocol described in the Fast® DNA Spin Kit for Soil (MP Biomedicals, LLC) handbook. The V6– V8 region of the bacterial 16S rRNA genes was amplified by polymerase chain reaction (PCR) using the universal bacterial primers 968-F-GC and 1401-R (Sigma-Aldrich, St. Louis, MO, USA). The PCR mixture contained 1 μL of each primer (10 ng μL−1 each primer), 25 μL of BIOMIX ready-to- use 2 reaction mix (Bioline, Ecogen), 2 μL of the extracted DNA, and Milli-Q water up to a final volume of 50 μL. The PCR thermo-cycling pro- gram consisted of 2 min of pre-denaturation at 95 °C, 35 cycles of RDP classification of the DGGE bands sequenced and corresponding matches (BLASTN) using the NCBI database with indication of the similarity percentages and sources of origin. The presence/absence of each band in each sample tested together with its intensity are also shown.

The DGGE analysis of the amplicons was performed with a D-Code Universal Mutation Detection System (Bio Rad Laboratories) using 8% (w/v) polyacrylamide gels with a urea/formamide denaturing gradient of 45 to 65%. DGGE running conditions were applied according to Roest et al. (2005). The gels were stained with GelRed Nucleic Acid Gel Stain (biotium) for 1 h. The most relevant bands were excised from the DGGE gel in order to identify the bacteria present in the sam- ples, resuspended in 50 μL of ultrapure water and maintained at 60 °C for 1 h to allow DNA extraction from the gel. A volume of 5 μL of the su- pernatant was used for reamplification with the original primer set. Be- fore sequencing, PCR products were purified with the GenElute PCR DNA Purification Kit (Sigma-Aldrich, St. Louis, MO, USA).

DGGE profiles were compared using the GelCompar IITM software (Applied Maths BVBA, Sint-Martens-Latem, Belgium). After image nor- malization, bands were defined for each sample using the bands search algorithm within the program. The peak heights in the densitometric curves were also used to determine the diversity indices based on the Shannon–Wiener diversity index. According to McDonald (2003) it ranges from 1.5 to 3.5 (low and high species evenness and richness, respectively).

Table 5.2 : RDP classification of the DGGE bands sequenced and corresponding matches (BLASTN) using the NCBI database with indication of the similarity percentages and sources of origin.

Phylum Deinococcus-thermus Class Deinococci Order Deinococcales Family Trueperaceae Genus Truepera	9		xx	xx	Uncultured bacterium (DQ345944)	99	UASB reactor
					Uncultured bacterium (KF911210)	99	Composting process
					Uncultured Thermus/Deinococcus group bacterium (HQ727577)	97	Petroleum-contaminated soils
	10	xx	xxx	xxx	Uncultured bacterium (DQ345944)	99	UASB reactor
					Uncultured bacterium (EU083501)	98	Hexadecane-degrading denitrifying consortium
					Uncultured Thermus/Deinococcus group bacterium (HQ727577)	97	Petroleum-contaminated soils
	11		xx	xx	Uncultured bacterium (KU648784)	99	Anaerobic full-scale reactors
					Uncultured bacterium (KM294370)	97	Sludge
	12		xx	xx	Uncultured Deinococci bacterium (HQ392846)	98	HRAPs treating piggery wastewater
					Uncultured bacterium (KU649843)	97	Anaerobic full-scale reactors
					Uncultured bacterium (FN598011)	97	Activated sludge
Phylum Actinobacteria Class Actinobacteria Subclass Actinobacteridae Order Bifodobacteriales Family Bifidobacteriaceae Genus Bifidobacterium	13	xx			Bifidobacterium longum (CP000605)	94	
Phylum Chloroflexi	14		xxx	xxx	Uncultured bacterium (KP641120)	92	High-rate denitrifying reactor treated with synthetic wastewater

Similarity indices were calculated from the densitometric curves of the scanned DGGE profiles by using the Pearson product–moment cor- relation coefficient (Häne et al., 1993). The taxonomic position of the se- quenced DGGE bands was obtained using the RDP classifier tool (50% confidence level) (Wang et al., 2007). The closest cultured and uncul- tured relatives to each band were obtained using the BLAST search tool at the NCBI (National Centre for Biotechnology Information) (McGinnis and Madden, 2004) (Table 5.2). Sequences were deposited in GenBank Data Library under accession numbers KY003166- KY003180.

5.2 Results and Discussion

5.2.1 Biotrickling filter performance

Anoxic biotrickling filter operation was carried for 222 days of operation with eight different steady state operation period. Within following BTF start-up, the removal efficiency of both readily biodegradable compounds of toluene (T) and ethyl-benzene (E) gradually increased and stabilized by day 2 of operation at 78 ± 6 and 91 ± 5%, respectively (Figure 5.3A and B), corresponding to elimination capacities (ECs) of 1.05 ± 0.18 g m^{-3} h^{-1} for toluene and 1.19 ± 0.22 g m^{-3} h^{-1} for E-benzene. On the contrary, hardly biodegradable compounds degradation was limited and a highly fluctuating xylene (X) removal was recorded during this first stage, with values as low as 2% increasing sporadically up to 44% (Fig. 2C). Similar to X, benzene (B) removal remained low and fluctuating at 10 ± 11% (Fig. 2D). The biodegradation of BTEX resulted in an average total CO_2 production of 5.8 ± 2.2 g m^{-3} h^{-1} throughout the first steady state operating period (Fig. 3). The pH value of the collected leachate rapidly increased after BTF start-up from 7 to 8.8 likely due to the denitrification in the biotrickling filter system.

By the end of stage I, a mass transfer test was performed by increasing ~ 2 times the BTEX inlet concentration in order to determine if the system was limited either by mass transfer or by biological activity. Inlet and outlet gas samples were hourly taken to analyze BTEX and CO_2 concentrations (Figure 5.4). The EC increase recorded for T, E and X as a result of the higher inlet load clearly showed that BTF performance was limited by the transport of these target compounds from the gas phase to the biofilm. Although adsorption in the packing material could initially contribute to the higher EC recorded, removal performance was maintained stabile throughout the mass transfer test and no negative removals due to TEX desorption was subsequently observed during the assay. This suggested the idea that all the compounds transferred were biodegraded in the biofilm, confirming the capacity of the microbial community to mineralize higher loads. In the particular case of benzene, the initial increase in EC observed (up to ×4.5) was followed by a removal deterioration (negative EC) when previous inlet concentrations were restored. This suggested a temporal benzene accumulation (either by adsorption in the biofilm or in the packing material or absorption in the recycling media) as a result of the higher concentration gradient during the mass transfer test, and a subsequent desorption of this compound before being biodegraded. Therefore, the limited biodegradation observed for B during stage I was in this case attributed to the accumulation of inhibitory intermediate metabolites as previously observed by previous studies during the aerobic biodegradation of BTEX (El-Naas et al., 2014; Trigueros et al., 2010).

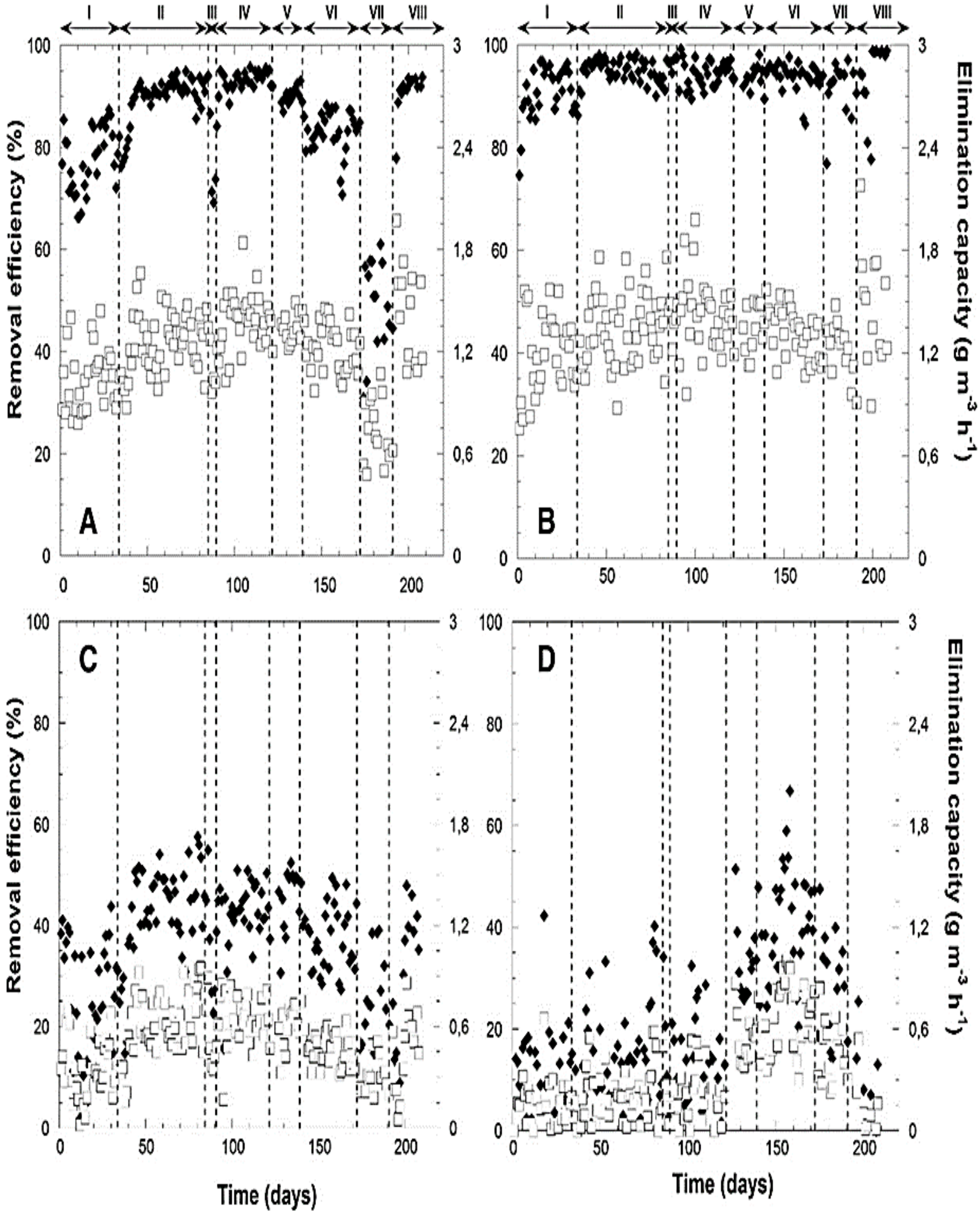

Figure 5.3 : Time course of toluene (A), ethylbenzene (B), xylene (C) and benzene (D) removal efficiency (♦) and elimination capacity (□). Dashed lines represent different operating stages as indicated in the upper part of the figure.

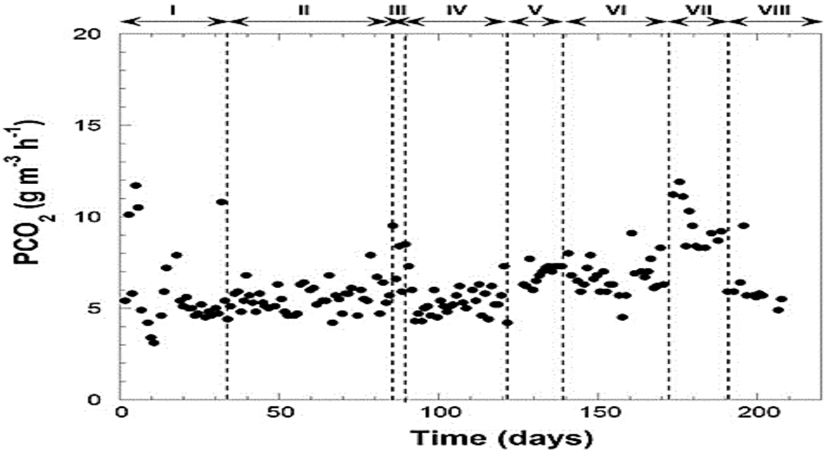

Figure 5.4 : Time course of CO_2 production. Dashed lines represent different operating stages as indicated in the upper part of the figure.

In order to overcome this particular limitation in biotrickling filter operation, the amount of MSM daily exchanged was increased by a factor of 3 from day 34 of operation. Increase by exchange rate at the new operating conditions resulted in a significant enhancement of the abatement performance for xylene, while in the meanwhile no improvement of benzene removal was observed during stage II. In this sense, toluene RE stabilized by day 42 at 91.4 ± 1.9%, E removal rapidly achieved a steady value of 94.9 ± 2.2% and xylene RE reached 45.2 ± 6.9% (~ 1.9 times higher than that recorded in stage) by day 40, resulted to ECs of 1.3 ± 0.2, 1.3 ± 0.2 and 0.7 ± 0.2 g m−3 h−1, respectively. It is worth noticing that, although previous works have reported similar T and E removal efficiencies than those here recorded (Lu et al., 2002; Rene et al., 2012; Wang et al., 2013), the ECs here achieved are lower than those observed during aerobic biofiltration of BTEX due to the lower inlet loads applied in this study (Balasubramanian et al., 2012; Chen et al., 2010; Gallastegui et al., 2011). In this sense, higher BTEX inlet concentrations are usually tested under aerobic conditions (up to 3 orders or magnitude higher than those found in O2-free petrochemical emissions, Torretta et al., 2015) and significantly lower EBRTs can be used due to the faster VOCs biodegradation rates commonly observed when oxygen is used as electron acceptor. Surprisingly the enhancement in pollutant removal in stage II did not result in a higher CO_2 production, which remained nearly stable at 5.5 ± 0.7 g m^{-3} h^{-1}. The constant PCO_2 was likely mediated by a higher amount of carbon being directed to the anabolic pathway for biomass production, that was decreasing the mineralization rate. On the opposite, no increase in benzene biodegradation was obtained after the increase in dilution rate, with a fluctuating value of 10.2 ± 11.6%. During stage II, the pH remained stable at 8.4 ± 0.1.

In this case, it is important to point out that previous studies have mentioned that neutral pH values promote higher benzene removal, and optimum biological activity for most BTEX degrading bacteria can be present at a pH between 6 and 8 under aerobic conditions (El-Naas et al., 2014; Hong and Lee, 2006). The effect of pH on BTEX biodegradation under anoxic conditions was then investgated by addition of a 6 N HCl into the liquid reservoir in stage III in order to reach a final value of 7.3 ± 0.1. This decrease in pH have detrimental effect over T and X biodegradation, and resulted with steady state REs during this stage of 77.0 ± 7.8 and 33.7 ± 13.1%, respectively. On the contray ethylbenzene removal was not significally affected by the pH change and remained at 96.0 ± 1.7%, while BTF operation at neutral pH resulted in a limited effect on B biodegradation, corresponding REs of 15.5 ± 12.2%. Due to the detrimental effect of the neutral pH on T and X abatement and the insignificant enhancement of B biodegradation, pH control was stopped after a week by day 90. During this stage, the CO_2 production experienced a noticeable increase reaching values of 7.8 ± 1.5 g m^{-3} h^{-1} as a result of the enhanced CO_2 mass transfer from the liquid to the gas phase under more acidic conditions.

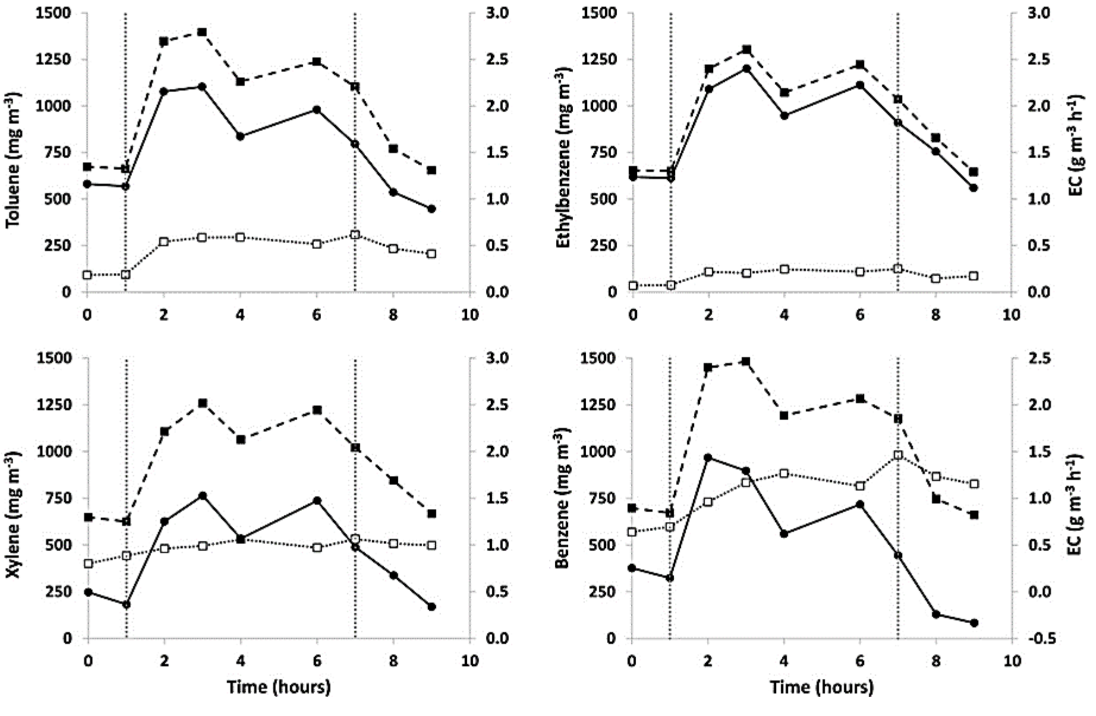

Figure 5.5 : BTEX inlet concentration (■, dashed line), outlet concentration (□, dotted lined) and elimination capacity (●, continuous line) during the mass transfer test.

After gave up the pH balance to 7, the previous T, E, X and B removal efficiencies were rapidly recovered once the pH was restored in stage IV (8.5 ± 0.1), with steady REs of 93.2 ± 1.7, 93.9 ± 2.4, 41.9 ± 6.8 and 9.8 ± 10.8%, respectively. Morever CO_2 production also stabilized at comparable values than those recorded during the second stage (5.3 ± 0.8 g m^{-3} h^{-1}). The BTF was operated under these steady state conditions until the 122th day of operation, when a 600 mL photoreactor was placed in the liquid recycling line interconnecting the 1.2 L-reservoir and the BTF column. The increase in the MSM volume only affected B removal performance, which had great impact the biodegradation and significantly increased up to 33.9 ± 8.6%, corresponding to an EC of 0.5 ± 0.2 g m^{-3} h^{-1}. E and X REs remained roughly stable between days 122 and 139 at 93.7 ± 2.2 and 45.2 ± 6.1%, respectively (corresponding to ECs of 1.4 ± 0.1 and 0.6 ± 0.1 g m^{-3} h^{-1}), while a slight decrease in T removal to 89.9 ± 2.0% was recorded (1.3 ± 0.1 g m^{-3} h^{-1}). The sustained abatement of benzene during the 17 days of stage V confirmed that the superior removal here recorded was not diretly associated to a merely benzene absorption in the liquid. The higher MSM volume likely act as a buffer reservoir of toxic metabolites, resulting in an enhanced biodegradation capacity of the microbial community. This also led to a higher PCO_2 during this stage, with an average value of 6.9 ± 0.5 g m^{-3} h^{-1}.

Previous works of authors on aerobic BTEX removal have pointed out the inhibitory effect of the secondary metabolites produced during pollutant biodegradation. For instance, toxic inhibitory metabolites such as catechol and methylated catechols have been identified as intermediates in the aerobic biodegradation routes of these pollutants (El-Naas et al., 2014). In addition, while the metabolites concentration in aerobic biodegradation systems is usually remained low at low inlet BTEX concentrations, previous observations suggest a higher accumulation of these interme-diates at similar inlet concentrations when working under O2 free anoxic conditions (Muñoz et al., 2013; Saucedo-Lucero et al., 2014). Other studies also highlighting a detrimental effect of feeding BTEX mixtures on the removal of a specific compound. Gallastegui et al. (2011) reported in their previous work the inhibition of xylene biodegradation when toluene was also fed to the biofilter. In the particular case of both X and B, their recalcitrant nature typically limits their degradation in biological systems.

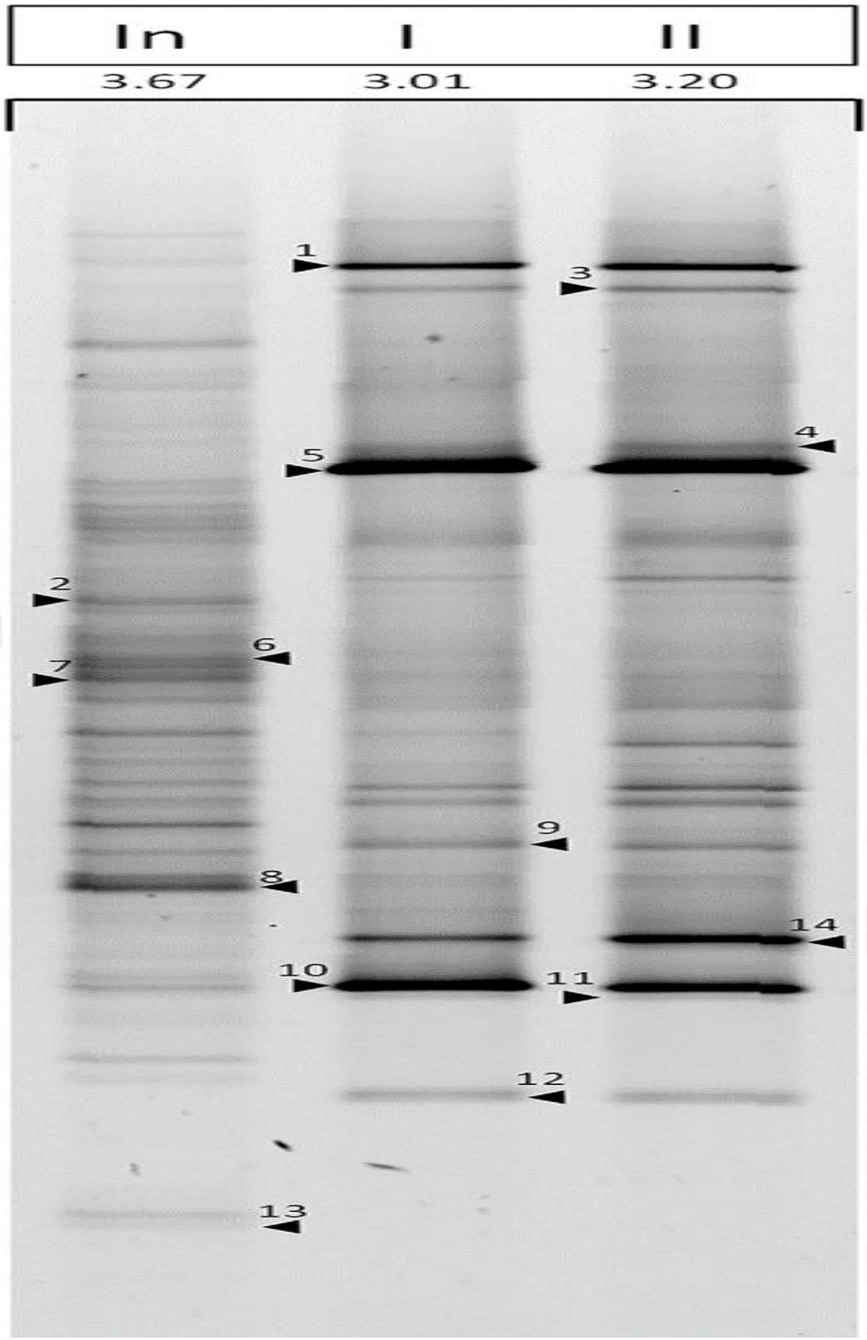

Figure 5.6 : DGGE profile of the main bacterial communities present in the inoculum (In) and the attached (1) and suspended biomass (2) by the end of the experimentation period.The Shannon-Wiener diversity indexes are indicated in the upper part of the gel. The sequenced bands are indicated by "►" and the corresponding number of each band.

At this point, a pretreatment stage based on UV oxidation might partially oxidize BTEX or their toxic metabolites into more biodegradable and less toxic intermediates such as acetaldehyde or formaldehyde (Moussavi and Mohseni, 2007). By day 139, the UV lamp was installed in the photoreactor to test its oxidation capacity in the liquid phase. While E removal was not affected by this modification, a slight decrease in T and X REs was recorded, reaching steady values of 82.6 ± 4.0 and 37.1 ± 6.6%, respectively. On the opposite, a gradual increase in benzene removal was observed during this stage, while achieving a maximum value of 66.8% (EC max = 1.0 g m^{-3} h^{-1}), with average values of 40.5 ± 10.5% and 0.6 ± 0.1 g m^{-3} h^{-1}. The differences in the chemical structure of these compounds usually led to the formation of by-products with different properties (such as water solubility) or biodegradability after their photolysis (Moussavi and Mohseni, 2007), which might explain the different effect on the biodegradation efficiencies recorded for each BTEX. PCO2 remained stable at 6.7 ± 0.9 g m^{-3} h^{-1}, and no pH change was either recorded, remaining at 8.5 ± 0.1.

By day 172, the UV system was removed from liquid line and installed in the inlet gas line to partially breakdown BTEX prior BTF feeding. Surprisingly, a deterioration in the abatement performance was observed for all BTEX compounds under this configuration. T was sensitive to UV lamp addition and biodegradation of T was significantly affected and the RE and EC decreased to 48.8 ± 8.7% and 0.7 ± 0.2 g m^{-3} h^{-1} respectively. Similarly, lower E, X and B removals were recorded, decreasing the corresponding REs to 91.7 ± 5.1, 24.5 ± 9.4 and 28.0 ± 10.2%, respectively. Surprisingly, CO_2 production significantly increased up to values of 9.3 ± 1.6 g m^{-3} h^{-1}. This result could be attributed to an increase in the mineralization rate due to the biodegra- dation of the by-products formed after BTEX photolysis, thus a higher amount of carbon was directed to CO_2 production in spite of the lower removal efficiencies recorded. The pH also decreased as a result of UV pretreatment and remained at 8.0 ± 0.1 during stage VII.

Lastly, the UV lamp was removed from the gas line by day 191 and the BTF was operated until day 208 as stand-alone treatment unit. Com- parable T, E and X abatement efficiencies (REs of 90.8 ± 4.4, 95.4 ± 3.9 and 33.6 ± 11.7%, respectively) to those recorded during stage II (under similar operating conditions) were immediately recovered. Conversely, almost no B removal was observed during this stage VIII.

5.2.2 Internal structure and molecular composition of microbial communities

The Shannon–Wiener diversity indices calculated from the bacterial DGGE gel were high for all the samples (this index often ranges from 1.5 to 3.5 (McDonald, 2003)), although a slight decrease in the diversity was observed in both the attached and suspended biomass at the end of the experiment (H = 3.01 and 3.20, respectively) compared to that of the inoculum (H = 3.67) (Fig. 5). Moreover, whereas the similarity coefficient between the attached and the suspended biomass was higher than 94%, nearly no similarity was observed between the inocu- lum and the attached and suspended microbial communities (lower than 4%). The particular conditions applied in the anoxic BTF likely pro- moted the development of a marginal percentage of the microbial com- munities (able to grow in oxygen free environments) present in the inoculum.

According to the RDP classifier tool, the 14 bands sequenced from the DGGE gel belonged to four different phyla (Fig. 5, Table 2): Proteobacteria (8 bands), Deinococcus-thermus (4 bands) Actinobacteria (1 band) and Chloroflexi (1 band). Proteobacteria was the predominant phylum, with the genus Dokdonella as the main representative (band 8). This genus is commonly isolated from soil or activated sludge, and many members from Dokdonella spp. are denitrifying bacteria (Yoo et al., 2009; Yoon et al., 2006). Bands 3, 4 and 5 belonged to the Alphaproteobacteria class, which have been previously described as tol- uene degraders using nitrate as the electron acceptor (Shinoda et al., 2005). Within the Proteobacteria phylum, two of the bands present in the inoculum (2 and 6) were not retrieved from the samples at the end of the experiment.

Bands 9 to 12 belonged to the Deinococcus-thermus phylum and were present in both the attached and suspended microbial communi- ties. Members of this phylum have been associated to the biodegrada- tion of aliphatic hydrocarbons and identified in air treatment bioreactors (Lebrero et al., 2015; Li et al., 2013; Militon et al., 2010). Blast search tool identified band 13 with the specie Bifidobacterium Longum, within the Actinobacteria phylum. Genus Bifidobacterium has been identified in microbial communities from activated sludge (Estrada et al., 2012), however it was only retrieved in the inoculum sample. Finally, microorganisms classified into the Chloroflexi phylum (band 14), which have been related to toluene biodegradation in a wide variety of biological systems (Fowler et al., 2014), were found in both final biomass samples.

6. CONCLUSION

In this thesis work odorous emissions from food fermentation and petrochemical industry were investigated with the aim of proper characterization and enhancing cost-effective and environmental friendly treatment from a technical perspective. The studies were carried out in two universities (Istanbul Technical University, Turkey and Valladolid University, Spain) and each industry branch was investigated in one university. The obtained resultes created a new challange to criteria selection for two-model sector emissions treatment.

Chapter 3 presents a complete characterization of a waste-gas emission from a real bakery yeast fermentation process. The chemical composition and the sensorial properties of this gas stream from bakery yeast process were determined for the first time in terms of both objective and subjective parameters. Instrumental analyses identified ethanol, acetaldehyde and acetone as the major malodorous VOCs in the emission, which reached a maximum concentration of 1181 mg m^{-3} during the first hours of the fermentation cycle. The odor concentration of the waste gas analysed via dynamic olfactometry resulted in an odor concentration of 39725 OUE m^{-3} that exceeded by a factor of 40 the odor legal limits and made compoulsory the proper management of fermentation process emissions. With this purpose, the study also identified the need to implement effective treatment technologies to abate these high concentrations of VOCs and odor prior discharge into the atmosphere. In this context, since biotechnologies represent the most sustainable alternative for the abatement of malodorous VOC at concentrations <2000 mg m^{-3}, a semi-pilot scale operation was succesfully implemented to treat ethanol, the dominant odorous VOC in bakery yeast fermentation emissions. The biofilter showed an almost complete biodegradation of ethanol even at concentrations higher than those found during peak emissions hour (RE of 97±1% and EC of 31.7 ±1.2 g m^{-3} h^{-1}), thus complying with legal regulations. Biofilter feeding with a waste gas containing ethanol, acetaldehyde and acetone resulted in a lower abatement performance compared to the treatment of individual

VOCs likely due to substrate interactions, where ethanol and acetaldehyde abatement were balanced at ~ 90 % and acetone was partially removed (70 %).

Chapter 4 evaluated for the first time the biodegradation kinetics and the interactions during the anoxic biodegradation of BTEX present as single compounds or in dual or quaternary mixtures, by a previously acclimated bacterial consortium. A complete set of kinetic parameters and their confidence intervals, calculated with the Monod model and a modified Gomperzt model, were provided. Results showed that both toluene and ethylbenzene were readily biodegradable by the bacterial consortium under anoxic conditions, with maximum biodegradation rates of 0.318 and 0.473 h^{-1} being recorded for these pollutants when degraded individually. However, the build-up of inhibitory intermediates in the liquid media resulted in a partial biodegradation of xylene and benzene when fed individually after long lag phase of 7 and 4.3 h^{-1} respectively, compared with toluene and ethylbenzene with a lag phase less than 1 h. The co-degradation of an additional BTEX decreased the specific pollutant biodegradation rates regardless of the dual BTEX mixture, which confirmed the occurrence of a competitive inhibition. These interactions had not been previously quantified under anoxic conditions prior to the current study. Whereas Monod model provided an accurate description of the biodegradation of toluene and ethylbenzene (R^2 ~0.97), the Modified Gompertz model supported better fit for benzene and xylene degradation (R^2 ~0.91). Furthermore, the Gompertz model accurately represented the inhibitory interactions between BTEX mixtures, although it failed in representing the degradation of the recalcitrant compounds when all four BTEX were present simultaneously.

The continuous biodegradation of BTEX under anoxic denitrifying conditions was tested in Chapter 6 for the first time in a biotrickling filter as an end-of-pipe technology for the abatement of O2-free BTEX emissions commonly found in the petrochemical industry. Toluene and ethylbenzene, the easiest biodegradable compounds, were efficiently removed (>90%) from the BTF startup, while the biodegradation of xylene was initially limited by the mass transfer of this pollutant from the gas emission to the liquid media. The low and unstable benzene abatement was associated to the accumulation of toxic intermediates. Their oxidation by a UV lamp installed in the recycling liquid line resulted in significantly higher benzene removals (up to 66.8%). Unexpectedly, the sequential coupling of the BTF with a UV-based pretreatment photoreactor did not result in an enhanced BTEX abatement performance but rather in the overall deterioration in the BTEX removal efficiency of the process. Despite the satisfactory REs achieved, the average ECs recorded (1.4, 1.5, 0.6 and 0.5 g m−3 h−1 for toluene, ethyl-benzene, xylene and benzene, respectively) were lower than those reported for aerobic biofiltration due to the lower BTEX inlet loads here tested. Finally, the particular features of the anoxic BTF here evaluated entailed a low similarity between the inoculum and the final microbial community developed, however a high diversity was always maintained throughout the entire experiment.

Based on the outcomes and limitations encountered throughout thesis study, further lines of research in the characterization and removal of food fermentation process should focus on:

- A continuous sustainability evaluation of new or upgraded air pollution treatment technologies for food fermentation process to overcome the lack of mass transfer limitation and detrimental effect of substrate interactions over biodegradation for multiple compounds.
- Scale-up the proper biological process to pilot and real scale applications.
- Improvement and optimization the dynamic olfactometry and instrumental measurement. Continuous evaluation of operational strategies depending on both viewpoint. Especially application of dynamic olfactometry is very new field and require intense research for case study application to proper use.

For the sector of petrochemical industry based on the results that obtained in this thesis work future studies should focused on:

- Determination of possible inhibitory by-products during O_2-free biodegradation of BTEX emissions.
- Process improvement the conditions for the recalcitrant compounds (B and X).
- New microbiological or technological approaches should be explored in order to improve mass transfer in biotrickling filters treating moderately (like BTEX) and highly hydrophobic pollutants.
- Selection of proper pretreatment alternatives to reduce the inhbitory effect of BTEX compounds over biodegradation.

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CURRICULUM VITAE

Name: İlker AKMIRZA

Place and Date of birth: İzmit (Turkey) in 09/12/1986

Citizenship: Turkish

E-mail: akmirzailker@itu.edu.tr

EDUCATION :

- **B.Sc. :** 2009, Yildiz Technical University, Civil Faculty, Environmental Engineering Department
- **M.Sc. :** 2012, Istanbul Technical University, Civil Faculty, Environmental Engineering Department

PROFESSIONAL EXPERIENCE AND REWARDS:

- 2011-2018 Istanbul Technical University (Istanbul, Turkey): Research and Teaching Assistant

PUBLICATIONS, PRESENTATIONS AND PATENTS ON THE THESIS:

Akmirza, I., Turker, M., Alp, K. (2018). Characterization and treatment of yeast production Process emissions in a biofilter. *Fresenius Environmental Bulletin 27,* 7099–7107.

*Car*vajal, A., **Akmirza, I**., Navia, D., Pérez, R., Muñoz, R., Lebrero, R. (2018). Anoxic denitrification of BTEX: Biodegradation kinetics and pollutant interactions. *Journal of Environmental Management 214,* 125–136.

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