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Abstract

The goal of this work was the treatment of polluted waste gases in a *bubble column reactor* (BCR), in order to determinate the maximum value of reactor's efficiency (RE), varying the inlet concentration (C_{in}) of the pollutants.

The gaseous mixtures studied were: (i) air with styrene and (ii) air with styrene and acetone. The liquid phase used to contain the biomass in the reactor was a *basal salt medium* (BSM), fundamental for the microorganisms' development.

The reactor used in this project consists of a glass column of 620mm height and inside diameter 75mm. In all essays there were continually measured: pH, dissolved oxygen and liquid's temperature. Temperature and pH were controlled (T=24ºC, $7.0 \leqq pH \leqq 7.7$). In all experiments the liquid volume (including the biomass) used in the reactor was kept constant (1.5L) as well as the total gas flowrate (1 L/min).

Concerning the goal of the work, some parameters were calculated: the organic load (OL), removal efficiency (RE), elimination capacity (EC), biomass concentration (x_f) and dry biomass concentration (Xd_w).

In a first series of experiments, the gas mixture used was air with styrene, varying its concentration from 191 $mg.m^{-3}$ to 6500 $mg.m^{-3}$.It was concluded that the RE maximum value (97%) was obtained for C_{in}^{Sty} = 4200 $mg.m^{-3}$. For the maximum tested value of C_{in}^{Sty}, RE obtained was 20%. In a second step, the gaseous mixture included acetone, varying C_{in}^{Sty} between 225 $mg.m^{-3}$ and 2659 $mg.m^{-3}$ and C_{in}^{Ac} between 153$mg.m^{-3}$ and 1389 $mg.m^{-3}$. The aim of these tests was the determination of C_{in}^{Ac} for which RE was maximum, obtaining C_{in}^{Ac} = 750 $mg.m^{-3}$. A third series of experiments was performed, in which C_{in}^{Ac} was maintained equal to that value and C_{in}^{Sty} was varied until higher values (5422 $mg.m^{-3}$). RE maximum values obtained in this last series were 100% for styrene and 40% for acetone.

One important conclusion is the fact that the microorganisms available degrade better styrene than acetone.

On the ambit of this study, it was possible to identify the species available in biomass: *Xanthobacter antotrophicus py2*, *Enterobacter aerogenes*, *Nocardia*, *Corynebacterium Spp.*, *Rhodococcus rhodochrous* e *Pseudomonas Sp*.

Resumo

O objectivo deste trabalho consistiu no tratamento de gases residuais poluentes num reactor biológico com borbulhamento do gás (BCR, *bubble column reactor*), a fim de determinar o valor máximo da eficiência do reactor (RE), variando a concentração desses gases na corrente de entrada (C_{in}).

As misturas gasosas estudadas foram: (i) ar com estireno e (ii) ar com estireno e acetona. A fase líquida usada no reactor para conter a biomassa era constituída por um meio mineral (BSM), fundamental para o desenvolvimento dos microrganismos.

O reactor usado neste projecto consiste numa coluna em vidro de altura 620mm e diâmetro interno 75mm. Em todos os ensaios eram medidos em contínuo: o pH, o oxigénio dissolvido, e a temperatura do líquido. O pH e a temperatura eram controlados ($7,0 \leqq pH \leqq 7,7$; T=24ºC). Em todos os ensaios, o volume de líquido (incluindo a biomassa) usado no reactor foi sempre o mesmo (1,5L), bem como o caudal da corrente gasosa (1 L/min).

No que concerne o objectivo deste trabalho, foram calculadas: taxa de carga orgânica (OL), eficiência de remoção (RE), capacidade de eliminação (EC), concentração de biomassa (x_f) e a concentração de biomassa seca (Xd_w).

Numa primeira série de ensaios, usou-se como corrente gasosa a mistura ar- estireno, fazendo variar a concentração deste de 191mg.m^{-3} a 6500 mg.m^{-3} Conclui-se que o valor máximo de RE (97%) foi obtido para C_{in}^{Sty} de 4200 mg.m$^{-3.}$ Para o valor máximo testado de C_{in}^{Sty}, o valor obtido da RE foi de 20%. Numa segunda série de ensaios, a mistura gasosa passou a incluir acetona, fazendo variar C_{in} S^{ty} entre 225 mg.m^{-3} e 2659 mg.m^{-3} e C_{in}^{Ac} entre 153 mg.m^{-3} e 1389 mg.m^{-3}. O objectivo destes ensaios foi determinar o valor de C_{in}^{Ac} para o qual a RE deste poluente fosse máxima, tendo-se obtido C_{in}^{Ac} = 750 mg.m^{-3}. Na última fase de ensaios, manteve-se C_{in}^{Ac} igual àquele valor, variando C_{in}^{Sty} até 5422mg.m^{-3}. Os valores máximos de RE obtidos neste conjunto de ensaios foram de 100% para o estireno e 40% para a acetona.

Uma importante conclusão é o facto de os microrganismos utilizados degradarem melhor o estireno do que a acetona.

No âmbito deste estudo, foi ainda possível identificar as espécies presentes na biomassa: *Xanthobacter antotrophicus py2*, *Enterobacter aerogenes*, *Nocardia*, *Corynebacterium Spp.*, *Rhodococcus rhodochrous* e *Pseudomonas Sp*.

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List of abbreviations and symbols

Ac- Acetone

C ^{Ac} – Acetone concentration (mg.m^{-3})

OL ^{Ac}- Acetone organic loading rate (g.m^{-3}.h^{-1})

V_f-air velocity (m/h)

BSM- Basal Salt Medium

X_f –Biomass (g.L^{-1})

Xd_m –biomass dry weight concentration (g.L^{-1})

BF - biofilter

BSC – bioscrubber filter

BTF- biotrickling filter

CAS- Chemical Abstracts Service

C- Concentration (mg.m^{-3})

DOC-dissolved oxygen content(mg/L)

EBRT- empty bed residence time (s)

V- Empty bed volume (m^3)

V_{evap}- evaporation volume (mL)

GC- gas chromatography

Q- Gas velocity (m/h)

C $_{in}$ ^{Ac}-inlet acetone concentration (mg.m^{-3})

C $_{in}$-inlet concentration (mg.m^{-3})

EC $_{in}$-inlet elimination capacity (g.m^{-3}.h^{-1})

C $_{in}$ ^{Sty}-inlet styrene concentration (mg.m^{-3})

H- level height (m)

H- liquid level height

m- mass (g)

M- molecular mass

OL- organic loading rate (g.m^{-3}.h^{-1})

C $_{out}$ ^{Ac}-outlet acetone concentration (mg.m^{-3})

C $_{out}$- outlet concentration (mg.m^{-3})

EC $_{out}$-outlet elimination capacity (g.m^{-3}.h^{-2})

C $_{out}$ ^{Sty}-outlet styrene concentration (mg.m^{-3})

EC $_w$- overall elimination capacity (g.m^{-3}.h^{-2})

OL $_w$- overall organic loading rate (g.m^{-3}.h^{-1})

V_t- Overall volume reactor (m^3)

PA- *Pseudomonas*

RE- Removal efficiency (%)

RA- Rose- Bengal

ST- standard

Sty- Styrene

C^{sty} – Styrene concentration ($mg.m^{-3}$)

OL^{Sty}- Styrene organic loading rate ($g.m^{-3}.h^{-1}$)

VOC- volatile organic compounds

V_g-volumetric flow (L/min)

V_L- working volume (mL)

1 Introduction

Since the Industrial revolution humankind is particularly concerned at new discoveries, which make life easier; consequently, different kinds of industries and technologies were developed. Unfortunately, this progress was accomplished with environmental negative aspects such as atmospheric pollution because of emanations from industrial plants (chemical, petrochemical, etc.) and vehicles. In order to contradict this tendency, it is crucial to develop measures for environmental protection and in the last decades there were being made many scientific and technical improvements in this area.

"Industrial and Manufacturing operations release, on a large scale, VOC to the air." [1] Volatile Organic Compounds (VOC) are very important for the environment as they contribute to the ozone depletion potential, creation of potential photochemical ozone, carcinogenicity, local nuisance from odour and global warming potential. [1] The selection of a particular technology or combination of technologies, for the treatment of VOCs, depends on such factors as: foul air flow rates, site characteristics including operation and preservation capabilities, treatment objectives, the characteristics and strength of air odors, and contaminant loading patterns. [2] New treatment techniques have been developed over the years, so that nowadays in developed countries, biological degradation of pollutants through microorganisms to treat the contaminated environment is being often used. One of the main advantages of biological degradation is a decline of biological wastes generation, compared to conventional techniques. *Xanthobacte*r, *Pseudomonas* are, among others, excellent examples of microorganisms very useful and suitable for VOC pollutants depletion and environment treatment, as these pollutants belong to the microorganisms' food chain. The compounds with the best biodegradability are the ones with low molecular weight, with simple bond structures and those that are highly soluble. "The contaminants are degraded into innocuous, less-contaminating products or all the way to carbon dioxide and water."[3] In this case, in order to prevent a climb of carbon dioxide (greenhouse gas) release as a result of VOC treatment that would contribute to the greenhouse effect and would lead to the global warming, biological methods are not appropriated to use in case of high VOC concentrations. [3]. In other words, these biological treatments are only strongly efficient and suitable in case of low VOC concentrations.

In the present work an experimental study is presented where the removal of two VOC from air: styrene and acetone, is performed in a biological reactor, at laboratory scale.

2 State of the Art

As previously referred the gaseous contaminants considered in this work were styrene and acetone. In this chapter some of their characteristics are described as well as the possibilities of their microbial degradation.

2.1 Pollutants characteristics

2.1.1 Styrene

Styrene is a colorless oily liquid with a water solubility value of 310 mg/L at 25ºC, which evaporates easily (its melting and boiling points are -30.6ºC (242.6 K) and 145ºC (418.15K) and doesn't dissolve appreciably in water. It´s soluble in organic solvents, it has a sweet odour at low concentration, but can have a sharp unpleasant smell at high concentrations [4, 5].

Figure 2-1 represents the chemical structure of styrene. The chemical and molecular formulas are, respectively, $C_6H_5CH=CH_2$ and C_8H_8 (M= 104.15 g/mol) its density, in liquid state is 0.906 g/cm^3 (T= 20ºC). The styrene Chemical Abstracts Service (CAS) number's 100-42-5 [6].

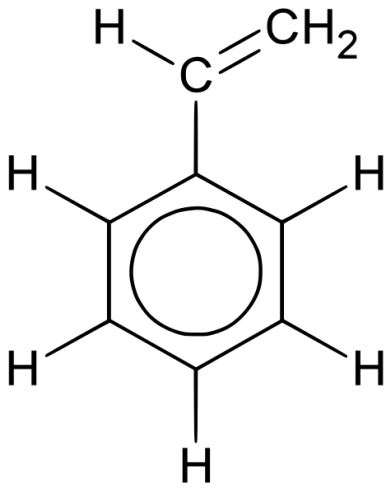


Figure 2-1- Chemical structure of styrene [7]

Styrene is a predominantly man-made chemical used for plastics manufacture, although low levels of this compound also occur naturally in a variety of foods such as fruit, vegetables, nuts and meats. General population exposure levels are usually orders of magnitude lower than occupational exposure levels. It is a commercially important chemical used in a wide variety of chemical processes, it´s the most used aromatic hydrocarbon in chemical industries, a basic building block for the plastic polystyrene, latex among others. Huge quantities of styrene are produced to make products such as rubber, plastic, resins, insulation, fiberglass, pipes, automobile parts, food containers and carpet backing. Styrene is a compound which easily liberates itself for the atmosphere. The atmosphere is the major sink for styrene losses and wastes. [8, 4]

Styrene is a VOC, which can contribute to the formation of harmful ground-level ozone and can enter the environment during the manufacture, use and disposal of styrene-based products. Significant sources of release are from emissions and effluents emitted during the production process and its use in polymer manufacture, e.g. adhesives and sealants industries. It may also be released from manufacturing processes, cigarette smoke, and stack emissions from waste incineration. There are also a few very minor natural sources of styrene in the environment. Styrene can be found in air, water and soil. It is quickly broken down in the air, usually within 1 to 2 days. It evaporates easily from soils and surface water, and is broken down by bacteria. It is not expected to build up in animals. It does not bind well to soils and may leach to groundwater, but its rapid breakdown minimizes this process. As a VOC it can be involved in reactions with other air pollutants that form ground-level ozone, which can cause damage to crops and materials as well as having potential effects on human health. [4]

2.1.2 Acetone

Acetone is an organic compound completely soluble in water at 20ºC. It is a colorless liquid with sweetish smell and a distinctive taste. The greatest danger regarding acetone use is that it poses a serious fire hazard. It has a flash point of -20ºC (253.2 K). The melting point is -95.4 ºC (177.8 K) and the boiling point 56.2ºC (329.4 K) at 1 atm.

Figure 2-2 represents the chemical structure of acetone. The chemical and molecular formulas are CH_3-CO-CH_3 and C_3H_6O (M= 58.08 g/mol). Its density in liquid state is 0.789 g/cm^3 (T= 20ºC).The acetone CAS number's 67- 64-1.

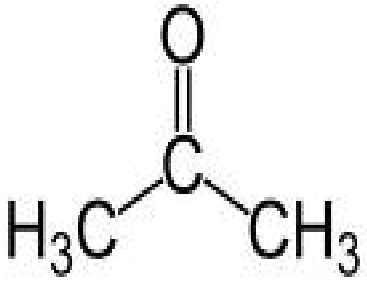


Figure 2-2- Chemical structure of acetone [9]

Acetone is the simplest and most important of the ketones. It is a colourless liquid with a mildly pungent and somewhat aromatic odour. It is primarily used as a chemical intermediate and as a solvent for cellulose acetate and nitrocellulose. It is used as a carrier for acetylene, and as a raw material for the chemical synthesis of a wide range of products such as ketene, methyl methacrylate, bisphenol A, diacetone alcohol, mesityl oxide, methyl isobutyl ketone, hexylene glycol, and isophorone. This compound is a mobile, flammable liquid that is miscible in all proportions with water and with organic solvents such as ether, methanol, ethyl alcohol, and esters. It is incompatible and reactive with oxidizers and acids. Containers of acetone may explode in a fire, producing poisonous gases. Acetone fires may be controlled with carbon dioxide or dry chemical extinguishers. Acetone undergoes many condensation reactions; in the presence of an amine, or ammonia, various esters condense readily with acetone. Acetone is considered a volatile organic compound by the U.S. Environmental Protection Agency. [10, 11]

2.2 Biological treatment of waste gases containing VOC

Gaseous emissions containing volatile organic compounds (VOC) may be treated by usual technologies, such as absorption, condensation, thermal and catalytic oxidation and active carbon adsorption. These physical-chemical treatments present some problems, including high costs and secondary wastes. [1] Furthermore some of these technologies present low selectivity and insufficient values of efficiency for some VOC removal.

“Biofiltration is the removal and oxidation of organic gases (VOCs) in contaminated air by beds of compost soil (biofilter media). “[12, 13] Biological treatment of waste gases and specifically of gases containing VOC is an attractive alternative, with several advantages when compared to chemical technologies. “Since the early sixties, biological processes have been introduced as a technique for odour abatement of waste gases.” [12, 13] Currently, these systems have a tendency of being used and developed all over the world, demonstrating their increasing importance as a great technique for air pollution control. [12, 13]

The biological technologies used for waste gases treatment are generally referred as “Biofiltration”. Biofiltration is a well established air pollution control technology successfully applied in a wide variety of applications to control odors and emissions containing VOC. Biofiltration consists in removing biologically gaseous contaminants by passing the carrier gas through a packed bed with aerobic microorganisms. Either the organic and inorganic air pollutants are degraded and transformed to innocuous products, such as water, carbon dioxide and biomass. These pollutants are used as unique carbon and energy sources and, consequently, biofiltration is limited by non toxic gases. [14]

Figure 2-3 shows the output of VOC biodegradation by the microorganisms in the biofilter. This process is naturally involved in an environment rich in oxygen, whereby the microorganism are classified as aerobic ones. [12]

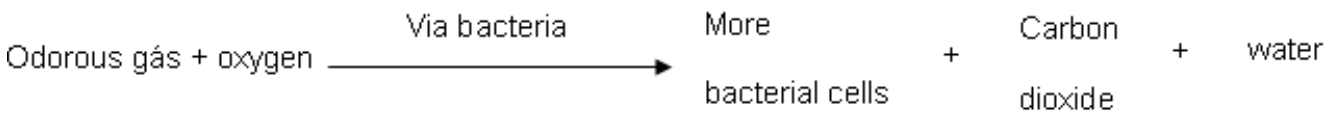


Figure 2-3- VOC degradation [2]

The efficiency of this process depends on various characteristics, such as inlet pollutant concentration, nutrient supplies and gas residence times (gas flow rate). Nutrient supplies must be securely provided by the biofilter media to the microorganisms. Residence time represents the amount of time the microorganisms are in contact with the contaminated air stream. Consequently, longer residence times produce higher efficiencies; however, the equipment must minimize residence time to allow the biofilter to accommodate larger flow rates. [15, 16]

Comparing air waste treatment biologically and chemically, it is possible to say that conventional techniques (adsorption, incineration and activated carbon) are more expensive than biological treatments. Biological treatment is environmentally friendly, generates depleted output products to be eliminated, it has lower operating costs and doesn't constitute a combustion source. It has a great stability on the steady-state and is performed at room temperatures. Moreover, biofiltration is a simple control system with restrictive energy consumptions.

The air waste biological treatment can be divided into three different technologies: bioscrubber, biofilter and biotrickling. "Biological treatment can be distinguished either by the mobility of the microorganisms and the liquid fase (as in trickling filter). In biofilters and trickling filters, the microorganisms are fixed in a support or a packing material."[17]

2.2.1 Reactors for biofiltration

2.2.1.1 Bioscrubbing

"Bioscrubbers (BSC) use counter current gas-liquid spray columns with microorganisms freely suspended in the aqueous phase."[18]

"A bioscrubber basically consists of a scrubber and a deposit vessel." [19] As soon the absorption process is over, the organic compounds of the waste gas must be removed from the absorbent. The major advantages of the absorbent regeneration are its influence on the cleaning efficiency and on the energy consumption of the absorber. This regeneration is carried out by the microbial species, which use the scrubbed gas compounds as a nutriment. [19]

There are several scrubber designs in the BSC system such as: tower packing, gas bubble, bottom column, spraying or nozzle scrubber. In this work, the biofiltration was developed and studied with the use of bubble column reactor. [20]

Next figure 2-4 represents an example of a design of the system and its parameters.

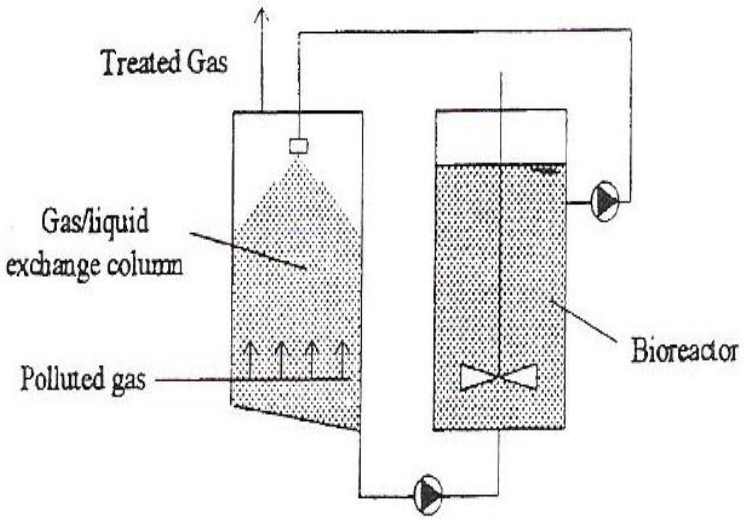


Figure 2-4- Design and control parameters [21]

In table 2-1, the main advantages and disadvantages of BSC are presented.

Table 2-1- Summary of the main characteristics of bioscrubbers for odour treatment biotechnologies [2]

Advantages	***Disadvantages***
➢ Bioscrubbing is more easily controlled because the pH, temperature, nutrient balance, and removal metabolic products can be altered in the reactor.	➢ Reliant on good gas dissolution, thus, it removes only highly soluble contaminants efficiently (gaseous pollutants with an air/water partition coefficient of less than 0.01)
➢ Removal of the products of pollutant degradation by washout (thus, avoiding of the biomass)	➢ Biomass growth has to be controlled to reduce solid waste output and to increase gas treatment efficiency.
➢ Acclimation capacity of the biomass provides efficient degradation of the pollutants.	➢ Controlled inputs of phosphate and potassium in the liquid media are required for efficient pollutant degradation, but this is not suitable for low concentration, wastewater treatment- generated odorants.
➢ Bioscrubbing is reliant on good gas dissolution, as it employs the absorption of pollutants into aqueous phase in a gas/liquid exchange column	
➢ The liquid phase bioreactor effluent is recirculated into the absorption column, providing excellent gas cleaning of highly soluble pollutants.	

One of the problems of this system is the sustained growth of the biomass that provokes the depletion of oxygen, arises the solid waste output and decreases gas treatment efficiency. In order to resolve this problem, there are several ways to reduce the biomass, such as: increasing the mean cell residence time, so that one can ensure the maintenance energy; confining nutrient supply, so that one can decrease efficiency of energy production for biomass growth.

2.2.1.2 Biofilters

"A biofilter (BF) consists of a filter-bed, traditionally composed of organic matter (peat, compost, sawdust, etc.), serving both as carrier for the active biomass and nutrient source. While flowing through the filter-bed, contaminants present in the polluted air are degraded by the active biomass. One important characteristic of the process is the absence of a mobile liquid phase as a consequence of which biofilters are suitable to treat poorly water-soluble pollutants."[21]

Next figure 2-5 shows an example of a design of this system and its parameters.

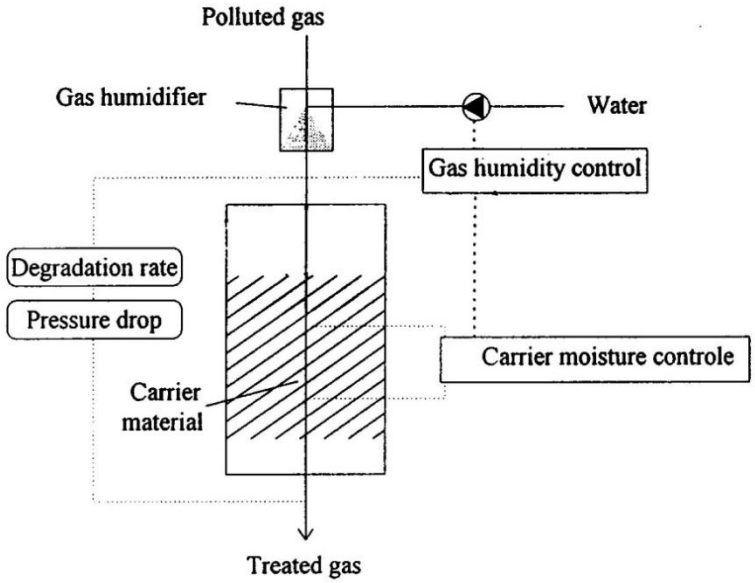


Figure 2-5- Design and control parameters [21]

Unlike the BSC system, there is only in BF system one stage where adsorption and biodegradation by microorganisms occur (column containing a material of immobilized microorganisms); the liquid phase is stationary (biofilm).

BF is often a high efficient and cheap system comparing with other atmospheric pollutants control technologies.

Table 2-2 summarizes the main advantages and disadvantages of biofiltration.

Table 2-2- Summary of the main characteristics of biofiltration for odour treatment [2]

Advantages	***Disadvantages***
➢ Simple, flexible design with low capital costs	➢ Design criteria still developing
➢ Good for treating high volumes of low concentration sulphurous odorants	➢ Large land area required
➢ 99% removal efficiency in streams containing aldehydes, organic acids, sulphur dioxide, nitrous oxides, and hydrogen sulphide	➢ >15 ppmH_2S can lead to rapid acidification of the biofilter media
➢ 90% removal of methane, propane, and isobutene.	➢ Dissolution of gas into liquid is the rate- limiting step, so long gas residence times are required
➢ Compounds with air/water partition coefficients of up to 10 can be treated in recirculating biofilters because the residence time 930-60s and specific surface area (300-1000m^2/m^3) are both high.	➢ Large media bed volumes are required to obtain such a long gas residence time, and operational control is limited, as there is no liquid phase involved.

2.2.1.3 Trickling biofilters

"Waste gas treatment in trickling biofilter (BTF) involves the use of a biological filter continuously fed with a liquid medium and packed with a synthetic carrier on which a biofilm grows." [21] The polluted gas passes through the carrier material, counter-currently to the mobile liquid phase, which ensures nutrient supply to the microorganisms. "Fresh medium fed to the reactor may be mixed with drain water recirculated to the system. "[21]

Next figure 2-6 shows an example of a design of this system and its parameters.

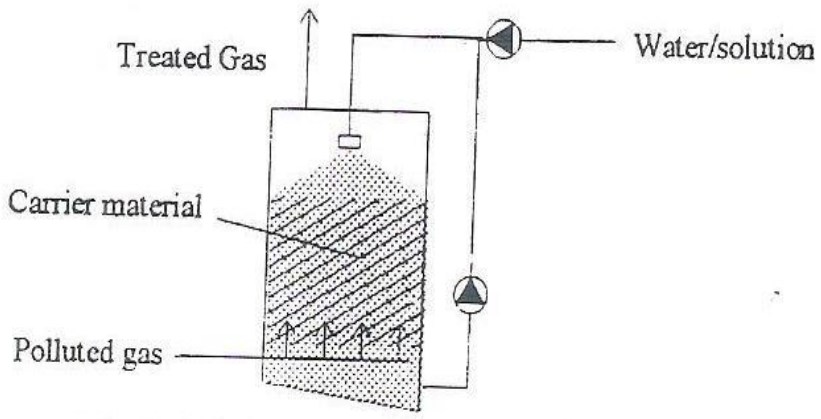


Figure 2-6- Design and control parameters [21]

BTF is a suitable and efficient technology for air treatment when working with high inlet loads of VOC. The BTF is an aerobic bioreactor composed by inert materials that can ensure a support for the biofilm. "In the BTF, a nutrient solution is trickled downwards trough the bed while the counter current undergoing treatment gases effluent flows upward". [22]

Like the BF system, there is in BTF system only one stage where adsorption and biodegradation by microorganisms occur (column containing a material of immobilized microorganisms); nevertheless, unlike the BF system, the liquid phase is not stationary.

"BTF present similar advantages to BSC: (i) easy control of biological process, (ii) easy elimination of reaction products by washing-out and (iii) good adaptation capacity of the active biomass." However BTF are not so frequently applied as BF systems nowadays. [21]

Table 2-3 summarizes the main advantages and disadvantages of BTF.

Table 2-3- Summary of biotrickling filters characteristics for odour treatment [2]

Advantages	***Disadvantages***
➢ Simple, flexible design	➢ Design criteria still developing
➢ Low capital costs, especially where existing trickling filters can be used	➢ Dissolution of gas into liquid is the rate-limiting step, so long gas residence times are required, necessitating recirculation of foul air.
➢ Acidification slower in nitrifying filters and with calcareous filter media (such as mussel shells- good buffering capacity to counteract acidification)	➢ Media require regular replacement (high costs)
	➢ Only approximately 60% hydrogen sulphide removal efficiency
	➢ Increased structure maintenance (corrosion concrete units).
	➢ Accumulation of excess biomass in the media bed reduces the specific surface area and bed volume and causes pressure drop, resulting in performance fall- off or requiring control techniques which compromise long- term performance.

In this biological technology referred as Biofiltration,, it is important to define the correspondent performance parameters, which are common to the different biological systems for treating contaminated air: organic loading rate (OL), removal efficiency (RE) and elimination capacity (EC), regarding a specific pollutant (VOC).

Equations 2.1 to 2.3 show how to evaluate those parameters for a bioreactor with liquid level height (H, m) where the gaseous stream to be treated enters the biofilter with a gas velocity (V_f ,m/h) and inlet concentration (C_{in}, $mg.m^{-3}$) of the pollutant considered and leaves it with outlet concentration (C_{out}, $mg.m^{-3}$)

$$OL(g.m^{-3}.h^{-1}) = \frac{V_f \times C_{in}}{1000 \times H} \quad (2.1)$$

$$RE(\%) = \frac{C_{in} - C_{out}}{C_{in}} \times 100 \quad (2.2)$$

$$EC(g.m^{-3}.h^{-1}) = \frac{V_f \times (C_{in} - C_{out})}{1000 \times H} \quad (2.3)$$

2.2.2 Microorganisms used for biofiltration

2.2.2.1 Styrene microbial degradation

Styrene is more and more used in chemical industry as a starting material in the production of synthetic polymers. It can also appear in the nature as a result of decarboxylation of cinnamic acid. It is very easy to transport styrene at low concentrations in the air. A major problem implicit to the transport of styrene is that styrene, as a toxic and carcinogenic gas, spreads easily and can prejudice human health. “In 1992, the annual worldwide production of styrene was estimated to be over 16 milion metric tons.” [23, 24] Gaseous styrene emission or effluent from industries and manufactures have been growing over the decades. Therefore, there are microorganisms in the nature that are capable to degrade styrene and are, thus, crucial to reduce the reported gas concentration in the air. [23, 24]

Some microorganisms can generate styrene, like *Pichia carsonni* which produces styrene from a ground fish decomposition product. [25]

Styrene degradation is possible with other microorganisms such as Xanthobacter Py2., *Pseudomonas sp*, *Nocardia sp., Enterobacter*, *Rhodococcus* and *Corynebacterium species* as well as the black yeast *Exophiala jeanselmei*. [24, 14, 26]

2.2.2.2 Aerobic styrene degradation

By using the *Pseudomonas sp.* for its biodegradation, it would be possible to remove styrene from industrial gases. However, there is little information about styrene microbial metabolism. The *Pseudomonas sp.* represents the best group capable of degrading styrene and produce styrene mono-oxygenase (very important for the degradation of styrene). [23, 11]

In order to get some information about styrene degradation, it was found in literature two references, which will be forwards described. The first reference says: “The only information about the organization of the styrene catabolic genes has been recently obtained for *Pseudomonas fluorescens* ST. This strain degrades styrene oxidation of its lateral chain and it has been shown that the upper pathway for the conversion of styrene to phenylacetate is encoded by four catabolic genes *styABCD*(…) The genes responsible for the catabolism of phenylacetate (lower pathway) remain to be investigated”. [23, 25]

This previous statement demonstrates the complexity of styrene catabolism and the lack of information about it. Figure 2-7 shows a scheme of styrene catabolism.

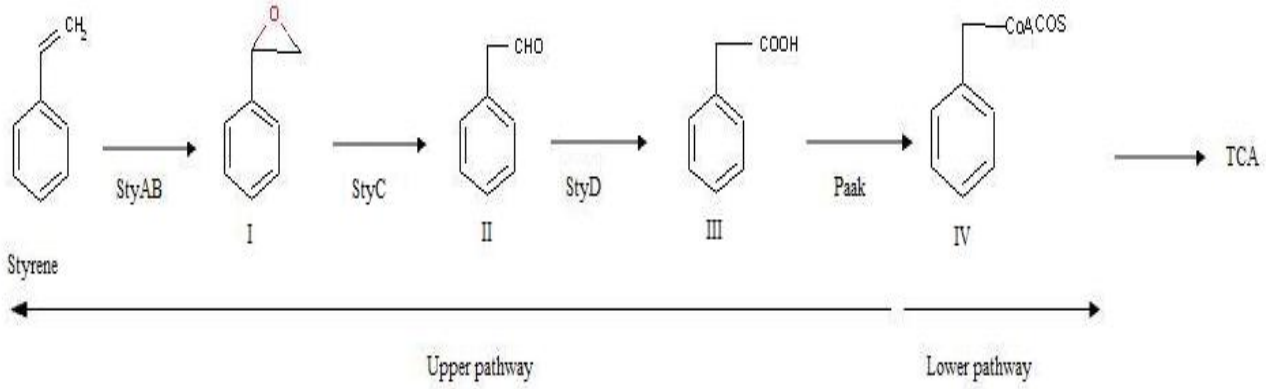


Figure 2-7- Pathway for the catabolism of styrene in *Pseudomonas Sp*. Strain Y2
I-epoxystyrene, II-phenylacetaldehyde, III- phenylacetate, IV- phenylacetyl- coenzimeA. Enzimes: StyAB, styrene monooxygenase; StyC, epoxystyrene isomerase; StyD, phenylacetaldehyde dedydrogenase; Paak, phenylacetyl- coenzyme A. [23]

The second reference is more comprehensive and clearer to understand as the first above. The aerobic degradation is described in two pathways: direct attack on the aromatic nucleus and an initial oxidation of the vinyl side- chain (figure 2-8). [26]

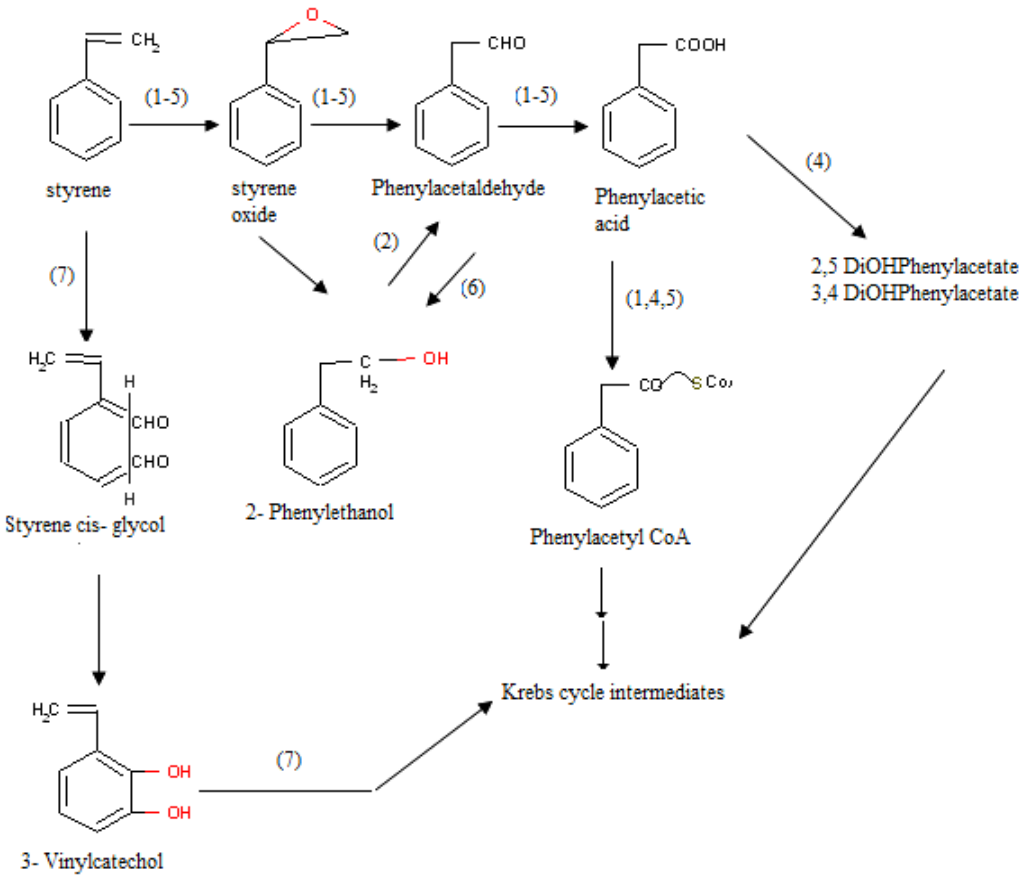


Figure 2-8- A summary of the major pathways of bacterial styrene degradation. The number(s) associated with each pathway identifies the organism(s) that have been shown to perform the particular transformation: 1, *P. putida CA-3;* 2, *Xanthobacter strain 124X;* 3, *Xanthobacter strain S5; 4, P. fluorescens ST; 5, Pseudomonas sp. strain Y2; 6, Corynebacterium strain ST-10; 7, Rhodococcus rhodochrous NCIMB 13259.* Dotted lines indicate proposed degradative routes yet to be formally demonstrated. [26]

- ✓ Side chain oxidation

"The side-chain oxidation pathway involves epoxidation of the vinyl side-chain by a flavin adenine dinucleotidependent, two-subunit monooxygenase followed by isomerisation of the epoxystyrene formed to phenylacetaldehyde (PAAL). This compound is subsequently oxidised to phenylacetic (PAA) (...). This conversion of styrene to PAA is generally referred to as the upper pathway of styrene degradation and appears to operate in many of the bacterial strains studied to date; examples include *Pseudomonas putida, Xanthobacter strain* 124X, *Xanthobacter strain* S5, *Pseudomonas fluorescences ST*, *Pseudomonas Sp. Strain* VLBI20 and *Pseudomonas Sp. Strain Y2*(…) The lower pathway is thought to involve the conversion of PAA to Krebs cycle intermediates but this has not been formally demonstrated in any styrene-degrading bacterium thus far."[26]

- ✓ Direct ring cleavage

Rhodococcus rhodochrous NCIMB 13259, a chemical dump isolate capable of growth on a diverse range of aromatic hydrocarbons, degrades styrene via direct oxidation of the aromatic nucleus.(…) An NAD^+-dependent *cis*-glycol dehydrogenase activity was detected in cells grown on nutrient broth and styrene. Cells grown under these conditions were also able to oxidise toluene *cis*-glycol.(…) 3-Vinylcatechol is further degraded via *meta*-cleavage to acetaldehyde and pyruvate.(…) Evidence of direct ring cleavage has also been reported in *P. putida* MST and *Xanthobacter strain* 124.(…)"[26]

2.2.2.3 Acetone microbial degradation

Acetone belongs to ketones class and is a toxic organic compound. Acetone vapors reduce oxygen concentration in the air, inducing a stifling and extremely explosive environment. When these vapors are inhalated, they can cause irritation to mucous membrane. Inhalation of higher concentration may cause a headache, nausea, confusion, drowsiness, convulsions and coma.

There is a variety of anaerobic and aerobic bacteria that grow up using acetone as a source of carbon and energy, in spite of acetone's toxicity. "While the biological production and consumption of these compounds has been well-documented, the bioquimical pathways and enzymes involved in their catabolism have only recently investigated."[28, 29, 30]

With regard to anaerobic catabolism, it “appears to involve an initial carboxylation of acetone to acetoacetate (…), followed by thiolytic cleavage to two acetyl-CoA residues.” Furthermore, “carboxylation reactions appear to play a role in a number of anaerobic transformations…”, although these reaction are to date not well known. In many bacteria such *as Desulfococcus biacutus*, *Rhodocyclus gelatinosus* among others, these reactions take place.

Towards aerobic catabolism, “for some aerobic bacteria, the metabolism of acetone has been proposed to proceed via an O_2^- and reductant-dependent hydroxylation reaction producing acetol-(1-hydroxyacetone) as the initial product.” It was found in literature that in aerobic enrichment cultures with acetone as substrate, “Gram-positive bacteria were isolated which attack acetone by oxygenase-dependent oxidation to acetol.”[30, 31]

There are even bacteria, such as *Xanthobacter* strain Py2, that are capable of degrading acetone either in aerobic and anaerobic metabolisms and are “capable of growth by using acetone as a source of carbon and energy”.[29]

2.2.2.4 Aerobic acetone degradation

The aerobic pathway of acetone degradation is shown in figure 2-7. Acetone is oxidized to acetol, then degraded to acetic acid or to pyruvate or to formic acid and finally to carbon dioxide and water. Various microorganisms can degrade acetone after initial carboxylation to acetoacetate, (mechanism shown in figure 2-8).

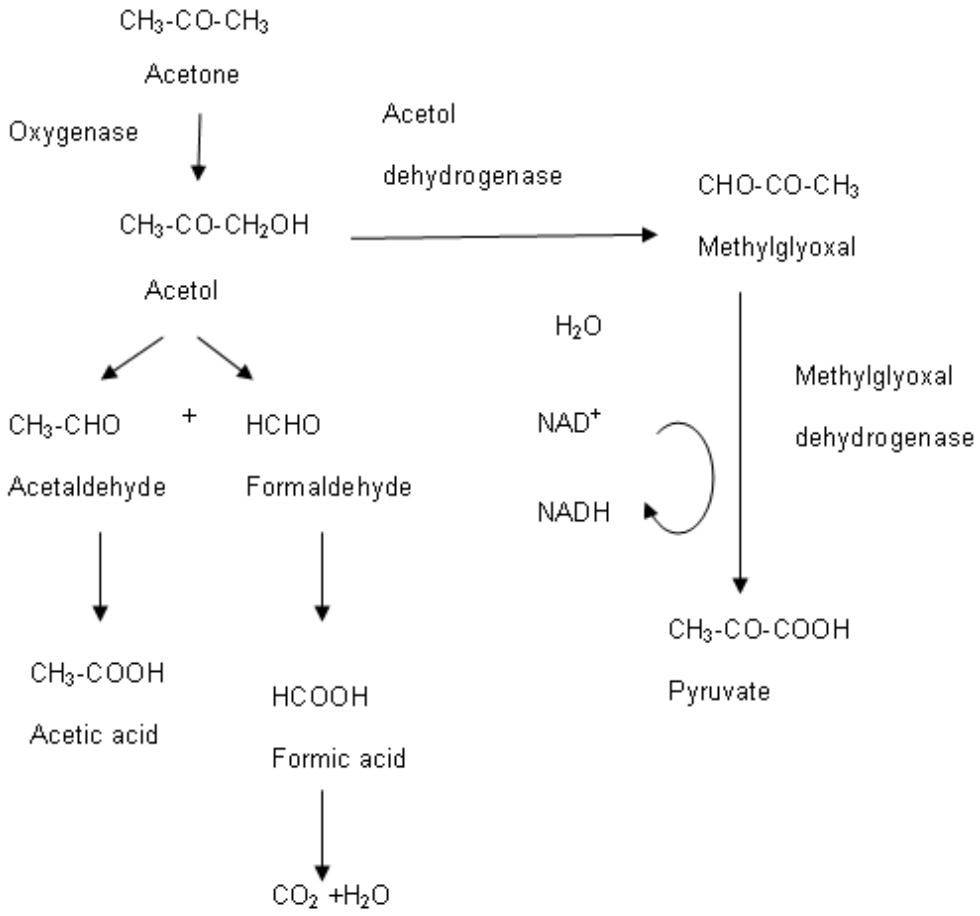


Figure 2-9- Aerobic degradation of acetone [2]

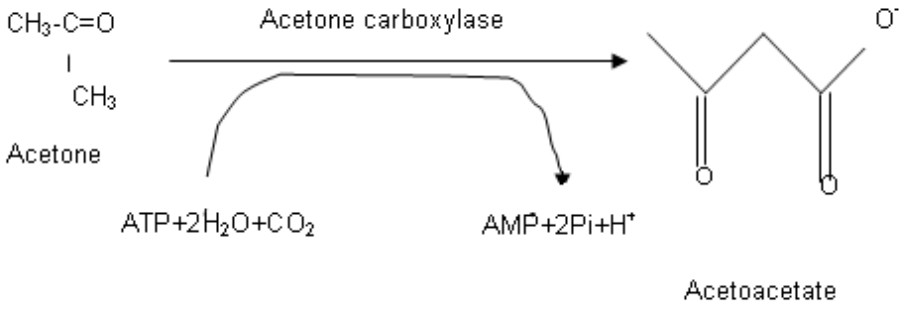


Figure 2-10- Carboxylation of acetone [2]

3 Experimental

3.1 Introduction

A bubble column reactor (BCR) was used to study the microbiological degradation of styrene and/or acetone in air streams.

. These two compounds, available as pure liquids (99% purity) were vaporized (separately or together) in air streams, in order to study the effect of VOC concentration. The liquid used for microorganisms culture and suspension was a basal salt medium (BSM), usually adopted in the laboratory, and described in table3-2. The microbiological species were discovered and identified through the preparation of different cultures, wherein in different species of microorganisms grow up. The identity of these species is described in “Results and discussion” section and the culture preparation is described in section 3.3.1.

A fixed volume of biomass suspension media (1.5 L) was used for all experiments. The total gas flow rate was kept the same in all experiments. The pH value of the suspension and the temperature were controlled and kept constant.

Three sets of essays were performed, in which the influence of concentration and composition of VOC mixture were studied (see section 3.4).

The monitoring of gas flow rate, pH, temperature (T), and dissolved oxygen was made continuously in each experiment.

In table 3-1 the range of values for the different variables and parameters are summarized for the three experimental parts.

Table 3-1- Summary of the experimental conditions

	Part I	Part II	Part III
pH	7.00- 7.70		
T (ºC)	24		
Volumetric flow (L/min)	1		
Gas velocity in the reactor (m/h)	13.6		
Empty Bed Residence Time (s)	84.8		
C_{in}^{Sty} (mg/m^3)	[191-3039]	[225-2659]	[681-5422]
C_{in}^{Ac} (mg/m^3)	0	[153-1389]	750

3.2 Experimental setup

The biological degradation system adopted for this work was a BSC, referred in this report as a bubble column reactor (BCR).

Figures 3-1 and 3-2 represent, respectively, the bubble column reactor (BCR) used in the experiments and the main components of the liquids feeding systems (basal salt medium and pure styrene and acetone). Figure 3-3 contains the schema of the experimental setup. Its legend also reports to some elements specified in Figure 3-2 by the same numbers.

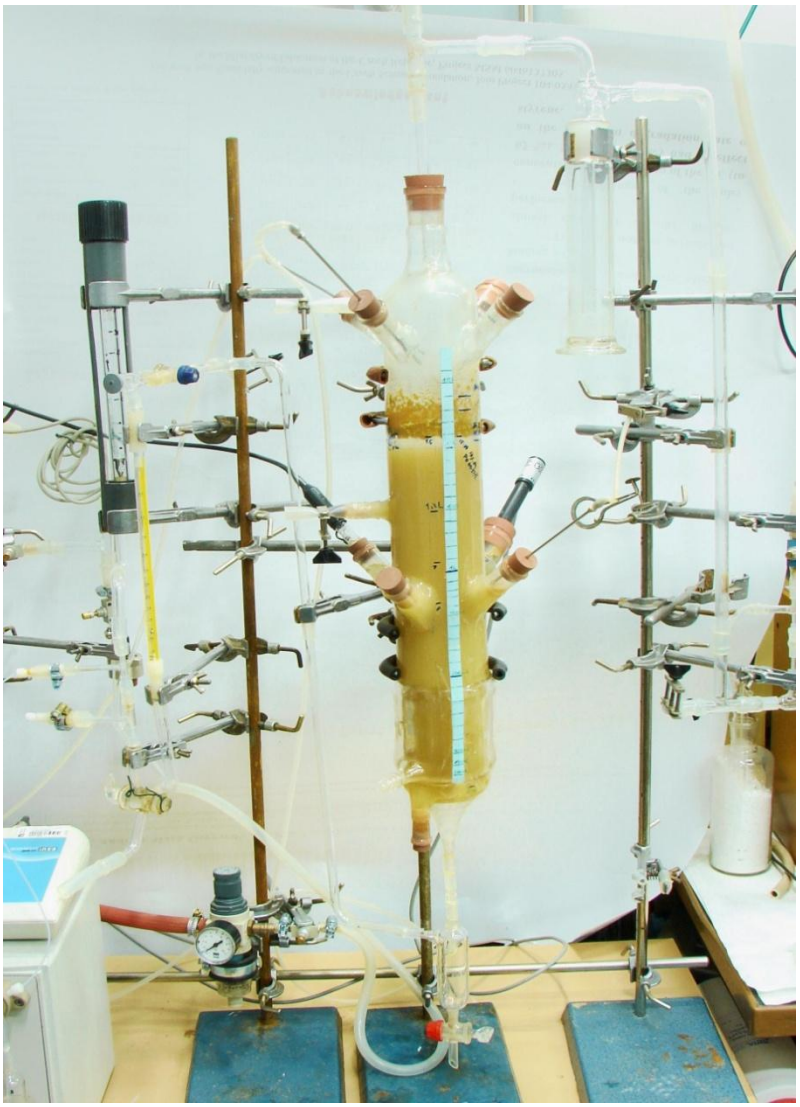


Figure 3-1- Bubble column reactor (BCR)

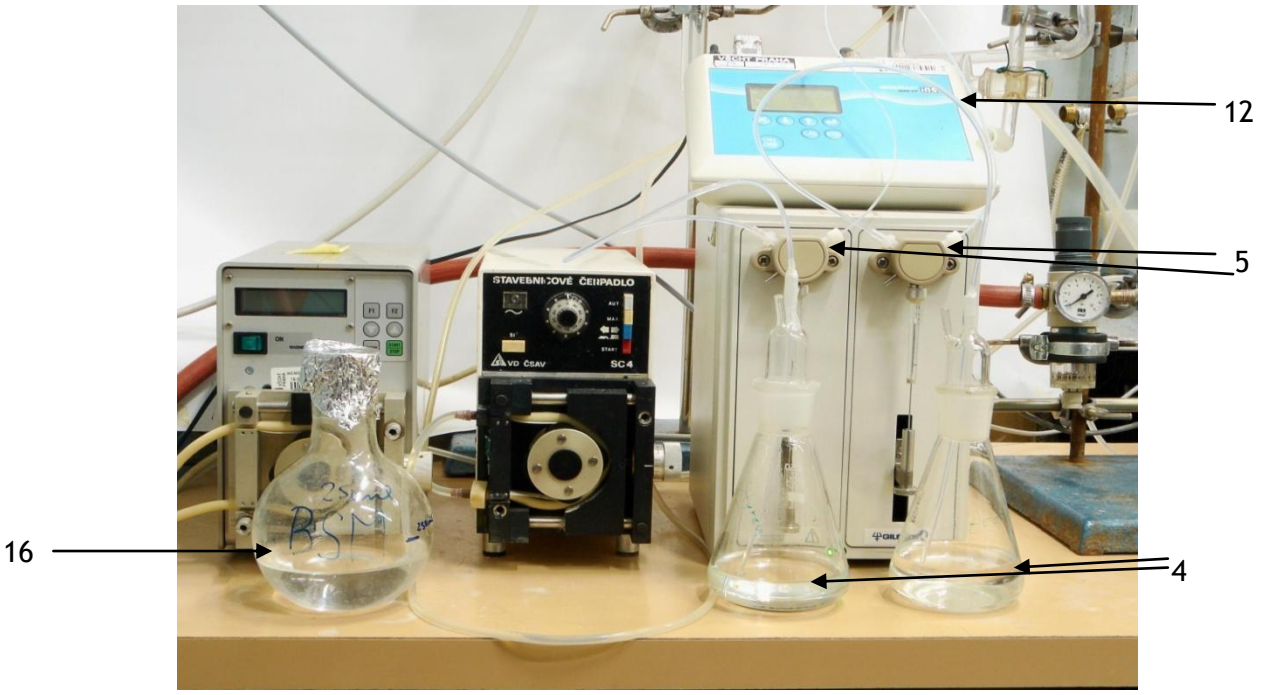
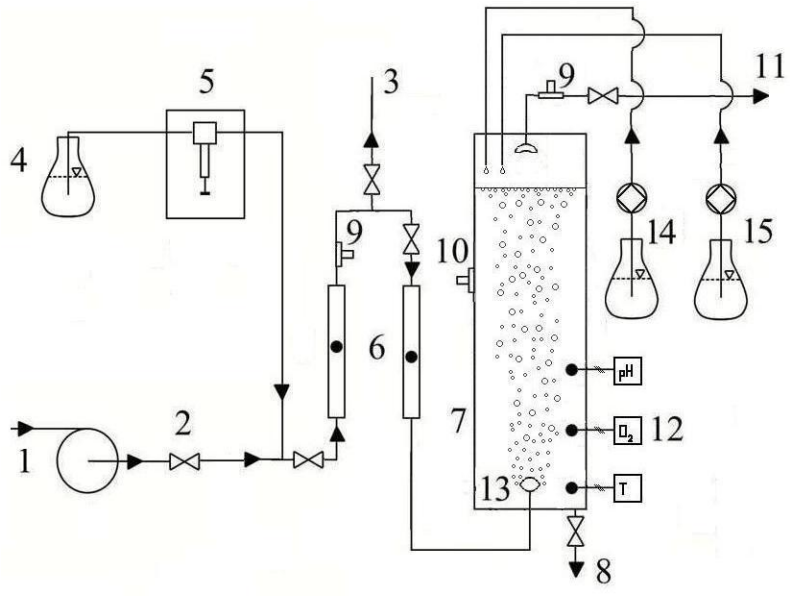


Figure 3-2- Feeding systems of basal salt medium (BSM) and pure styrene and acetone



1-air inlet; 2- air flowrate control valve; 3-flow regulation, 4- pure styrene or acetone; 5- syringe pump, 6- flowmeters, 7- reactor,8- drain valve, 9-gas sampling point ,10- liquid sampling point,11- gas outlet 12- sensors (pH, O_2, T) ,13- air distribution, 14- potassium hydroxide solution, 15- BSM solution.

Figure 3-3 - Schematic representation of the experimental apparatus

The bioreactor used is a glass cylinder with 75mm internal diameter, 620 mm height and 0.442 dm^2 crosscut area.

As it can be seen in Figure 3-3 the gas inlet is situated in the bottom of the BCR. The gas stream is obtained (at the desired composition) by mixing air with vaporized acetone and/or styrene (4, 5) and the gas flowrate is adjusted by means of control valves (2, 3, and 10) and flowmeters (6)

The BCR contains one sampling point (10) for the biomass suspension, situated at 25 cm height.

The addition of liquids (BSM solution and reagents for pH adjustment) is made in the top of the reactor. For the addition of BSM a STAVEBNI COVÉ CERPABLO, pump was used; for acetone and/or styrene a GILSON V= 100 µL, SYRINGE PUMP 402. The solution of KOH was fed to the BCR by means of a pump STAVEBNI COVÉ CERPABLO SC4

The BCR has also placings for some sensors for the pH, dissolved oxygen and temperature. The sensors used in this study were: electrode MPH 66 Insa was set 7, 0±0.1 for pH, MULTIMET INSA MFD 77 for the dissolved oxygen and MULTIMET INSA for the temperature.

The BCR operation was automatized and all the monitoring and control equipment were connected to a computer, where specific software allowed the changing of operating conditions and the online monitoring of all important parameters and variables.

Two sampling points for gas were available, for BCR inlet and outlet streams.

3.3 Experimental procedure

3.3.1 Biomass

One of the main elements for the experimental study was the biomass needed for VOC degradation. The cells used for starting the bubble column reactor (BCR) came from another biological reactor in the laboratory. This biofilter (BF) has been working during the last 3 years with streams of air, styrene and acetone.

The microbial cultures for the BCR were prepared in Erlenmeyer flasks with mineral medium (BSM) and further mixed in a rotary shaker.

3.3.1.1 Basal Salt Medium (BSM)

The composition of the culture media (BSM) used in the experiments is described in table 3-2. The composition of the trace elements (V= 50µL) is represented in table 3-3.

Table 3-2- Composition of the basal salt medium (BSM)

Component	Concentration (g/l)
$(NH_4)_2SO_4$	1,5
KNO_3	0,5
K_2HPO_4	4,3
KH_2PO_4	3,4
$MgCl_2.6H_2O$	0,34
Trace elements	See table 3-3

Table 3-3- Composition of trace elements, V= 50µL (BSM)

Component	Concentration (g/l)
$FeSO_4.7H_2O$	1,5
$ZnSO_4.7H_2O$	0,5
$MnSO_4$	0,1
$CuSO_4$	4,3
$COCl_2$	3,4
$NaBO_2$	0,02

3.3.1.2 Characterization of microbiological species present in the BCR

The characterization of biomass was performed twice, during all the experimental study, at the end of Part I and at the end of Part II (see Table 3-1).

In order to identify and quantify the species present in the microbial medium used in the BCR, some cultures were tested using different media as follows:

RB: *ROSE BENGAL CHLORAMPHENICOL AGAR,* with 100 mg/l chloramphenicol. The microorganisms developed in this culture are only eucariotes.

PA: *PSEUDOMONAS AGAR BASE,* with cetrimide 10.0 mg/l and cephalosporin 50.0mg/l).

ST: *STANDARD PLATE COUNT AGAR (APHA).* This culture allows determining the total number of microorganisms in the reactor.

AGAR BACTERIOLOGICAL (AGAR NO 1) (LP 11 B). This is a clean culture, for which the gaseous components being studied (air+styrene: BSM Sty, air+acetone: BSM Ac and air+ styrene+ acetone: BSM Ac+Sty) were used as substrate for microbiological growth.

For each characterization, a 5ml sample was taken from the liquid in the reactor (BCR) and it was mixed with 25ml of BSM. Afterwards, all the volume (30 ml) was placed in a test tub to prepare different dilutions as presented in Table 3-4. The cultures were kept in a laboratory for two days at 25ºC.

Table 3-4- Number of samples analyzed for the different cultures and different dilutions

	100	1000	10000	100000
RB	1×	2×	1×	
PA	1×	2×	1×	
ST		1×	2×	1x
BSM Ac	1x	2×	1×	
BSM Sty	1×	2×	1×	
BSM Ac+Sty	1×	2×	1×	

Besides the characterization described above, during the experimental study the biomass used in the BCR had to be monitored. Several measurements of optical density and dry mass were made (see section 3.5) in order to evaluate the biomass concentration and make some adjustments wherever needed.

3.3.2 Experiment description

The experimental apparatus was automatized and its operation was possible by using specific computer software.

At the beginning of the experimental essays of VOC degradation, the prepared biomass suspension (1.5 L) was taken from the rotary shaker and added to the BCR, keeping the liquid level in the reactor (without gas bubbling) at 0.32 m. The values of BCR operating temperature and pressure were constant (24ºC and 1 atm) as well as the total gas flowrate (1 L/min). Using the computer software, the desired inlet gas composition for each experiment was established.

The stabilisation of inlet gas stream composition took some time and several samplings and analysis by gas chromatography were made until a stable value was obtained (see section 3.4.1). At this time, sampling of the outlet gas was started in order to analyse its composition (see section 3.4.1)

The experiment was run until the stabilization of the outlet gas composition. The stabilised values were registered.

Meanwhile, all the important parameters were monitored and their values adjusted if necessary. The pH value was corrected by addition of KOH solution and more rarely by a few drops of acid.

As already referred, a first series of experiments was planned with mixtures of air and styrene (Part I, in table 3-1). From one experiment to the following only the inlet gas composition was changed. All other parameters were kept constant. The experiments were run sequentially with increasing concentrations of styrene. At the end of this series, the BCR was “cleaned” until the styrene level at the outlet gas came to zero. The first set of experiments took place in about 30 days.

For the second series of experiments, mixtures of air, styrene and acetone were used, in the same conditions as described before (Part II, in table 3-1).

In the final set of experiments (Part III in table 3-1), mixtures of air with styrene and acetone, with constant acetone inlet concentration (C_{in}^{Ac}= 750 mg.m^{-3}), were studied.

The biomass in the BCR was taken out from the reactor and centrifuged whenever need. This was evaluated by its visual aspect and the results of optical density measurements.

The quantity of acetone in the liquid was also evaluated by HPLC a few times in the last period of experiments (See section 3.4.2).

3.4 Analysis of VOC

3.4.1 VOC in gas streams

The analytical method used for VOC determination in gas streams was gas chromatography. The equipment used was a gas chromatograph (Hewlett Packard 6890 N series, California, USA) with a flame ionization detector FID, figure3.4. Gas sampling was performed at the BCR inlet and outlet (figure 3-5) by means of a chromatographic syringe (Microliter Syringes gastight H 1750, V= 500 µL, Hamilton CO, Reno, Nevada) and detection temperatures was 250 ºC.

Figure 3-4- Gas chromatograph (Hewlett Packard 6890 N series, California, USA)

-

Figure 3-5- Injector (Microliter Syringes, Hamilton CO, Reno, Nevada)

3.4.2 Determination of acetone in liquid medium (BSM)

As already referred (3.3.2) in specific cases and in order to complete the evaluation of the bioreactor performance, and particularly the acetone retention time in the BSM before degradation, it was necessary to measure the concentration of acetone in the liquid medium. The analytical method chosen was High performance Liquid chromatography.

The liquid BSM samples from the BCR were centrifuged (Biofuge stratus, HEREAUS) and further analyzed by HPLC (RP- HPLC_ DELTACHROM WATREX), for determination of the quantity of acetone.

The analytical conditions are presented in table 3-5,

Table 3-5- Conditions for acetone determination by chromatography (HPLC)

System	*DELTACHROM WATREX PRAHA S.R.O., Czech Republic*
Pump	DELTACHROM SDS 030
Detector	UV 6000LP (DIODE ARRAY DETECTOR)
Auto sampler	BANI MARATHON, SPARK
Software	CHROMQUEST
Mobile phase	Methanol: demineralized water 3:2
Column	250x 4 mm NUCLEOSIL 120-5 C18
Pressure working	3000-4000 psi
Flow phase mobile	1 $mL.min^{-1}$
Acetone retention	3.2 min
Wavelength	268 nm

3.5 Characterisation of the BCR biomass

3.5.1 Optical density

One must be ensured that the biomass doesn´t turn out to be too much opaque, because that means that the biomass has an excessive organic load and, consequently, a very poor presence of oxygen causing system's anaerobiosis. In those conditions, the microorganisms can degrade badly the pollutants or even begin to degenerate. Thereby, the biomass optical density determination is very important to decide if it is necessary to make a centrifugation, in order to decrease organic load and avoid anaerobiosis.

A sample of the biomass of the reactor was collected for measuring the optical density by spectrophotometer (Spekol 11 Labo_MS, spol s.r.o). The sample was set in cuvettes and the wavelength of spectrophotometer used was 400 nm. When necessary, the sample was diluted for consecutive determination of its concentration from absorbance value.

3.5.2 Dry mass

A sample of the biomass (20.00 ml) of the reactor was centrifuged (BIOFUGE STRATOS HERAEUS) during 20 minutes under 10000 rotation/min, twice. After the first centrifugation, the liquid was removed and replaced by distilled water.

After the second centrifugation, the solid phase was separated and placed in a volumetric flask (10.00 mL) and the 10.00 mL were completed with distilled water.

This biomass solution was placed in 9 platinum dishes, previously weighted. The dishes were put in a drying oven (ZSK-1, KCW 100) for 2 hours (60 min at 70ºC and 60 min at 105ºC). After drying and back to ambient temperature (in an excicator), the dishes were weighted, for dry mass quantification.

4 Results and discussion

4.1 Overview

In this chapter the results obtained in the different parts of the experimental study and their further discussion are presented.

Sections 4.1.1, 4.1.2 and 4.1.3 are related to Part I (air+ styrene), Part II and Part III respectively. The 4.1.4 and 4.1.5 sections present the evaluation of the biomass compared with dry mass and the microbiology species, respectively.

The experimental values registered in all experiments are available in Annex I.2

As referred in chapter 3, all experiments were run with total gas flow rate kept constant (1 L/min, 24ºC). The gas velocity in the BCR was then 13.6 m/h and the EBRT 84.8 sec. The volume of biomass was also constant and equal to 1.5 L.

4.1.1 Styrene degradation in the bubble column reactor (Part I)

The main goal of these experiments was to find out the inlet styrene concentration (C_{in}^{Sty}) when removal efficiency (RE) is maximum: as RE reaches a peak and starts to decay, it is possible to identify the value of C_{in}^{Sty} responsible for that maximum. Furthermore, there were also calculated the styrene organic loading rate (OL^{Sty}) and styrene elimination capacity (EC^{sty}): the first variable is very important to evaluate and control the aerobic/anaerobic environment of the system; the second variable expresses the removal of styrene quantity from air stream. EC^{sty} and RE are, then, interrelated.

The values of OL^{Sty}, RE, and EC^{sty} are obtained by equations 2.1, 2.2 and 2.3, respectively.

In figures 4-1 to 4-6, there are represented several results related to styrene degradation; in Annex I.2 it is possible to find the corresponding tables.

In figure 4-1 it is possible to see the evolution of inlet/outlet concentrations of styrene in time and to compare these with OL^{Sty} values obtained along the experimental period (30 days).

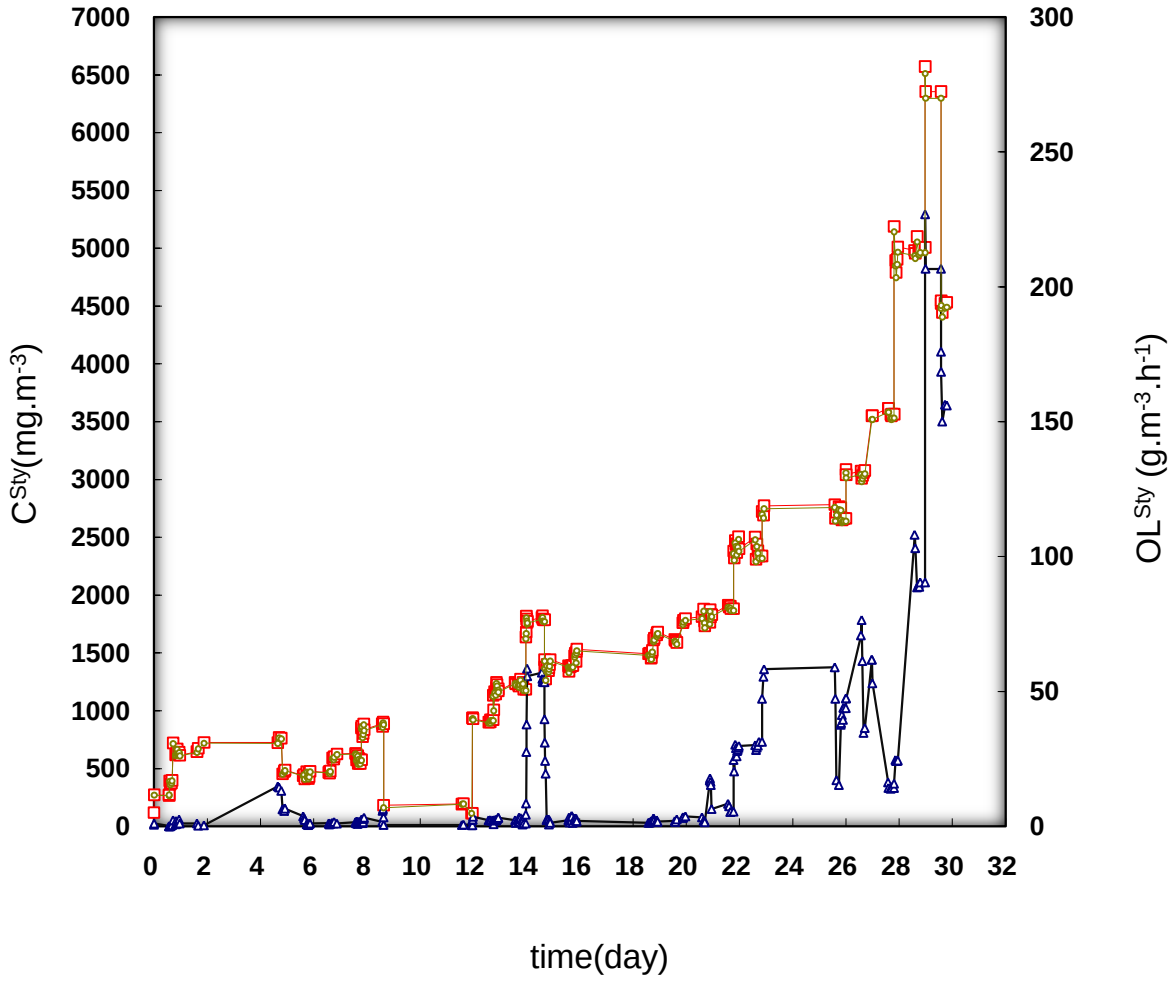


Figure 4-1 –Inlet, □ and outlet, Δ, concentrations(C^{Sty}), organic load rate (OL^{Sty}), ○ for styrene in time (day)

Each experiment is then represented by a group of three marks (□, Δ and ○). In each day a variable number of experiments could be performed. As it can be seen in figure 4-1 the styrene inlet concentration (C_{in}^{sty}) was gradually increased from 250 to 6500 $mg.m^{-3}$. Until the 19th day, C_{in}^{sty} has increased slowly (with exception for days 9 and 12, when smaller C_{in} values were tested); however, from day 20, it has increased rapidly. As one can see through the graphic, the maximum C_{in}^{sty} values were used on day 30 and the minimum values were tested between the days 8 and 12.

The OL^{sty} and C_{sty}^{in} vary at the same ratio, and obviously OL^{sty} values follow the same behavior as that of C_{in}^{sty} along the several days. The maximum values of OL^{sty} (between 250 and 300 $g.m^{-3}.h^{-1}$) corresponds to C_{in}^{sty} between [6500-7000] $mg.m^{-3}$.

The range of OL ^{sty} values studied was at an interval of values acceptable, according to the literature (Hanna et al.) [8]. This fact is important, as it indicates that the microorganisms can degrade styrene in a normal aerobic environment (the microorganisms are exclusively aerobic in this study); on the contrary, if OL ^{Sty} values exceeded a specific value-limit, then oxygen wouldn´t be completely available in BSM liquid, the system would become less aerobic, and consequently, microorganisms would degenerate with the lack of oxygen , what could cause drastic consequences for RE and EC values, reaching rapidly minimum values.

Another important aspect to emphasize is the experience duration of thirty days. The main criterion was the outlet styrene concentration (C_{out}^{Sty}) increasing in air stream: the less the microorganisms can degrade styrene, the more styrene is present in air stream. As one can observe, on day thirty C_{out}^{Sty} is very high, expressing that microorganisms already had begun to degenerate.

Figure 4-2 represents the evolution of styrene inlet concentration (C_{in}^{Sty}) en removal efficiency (RE) in time

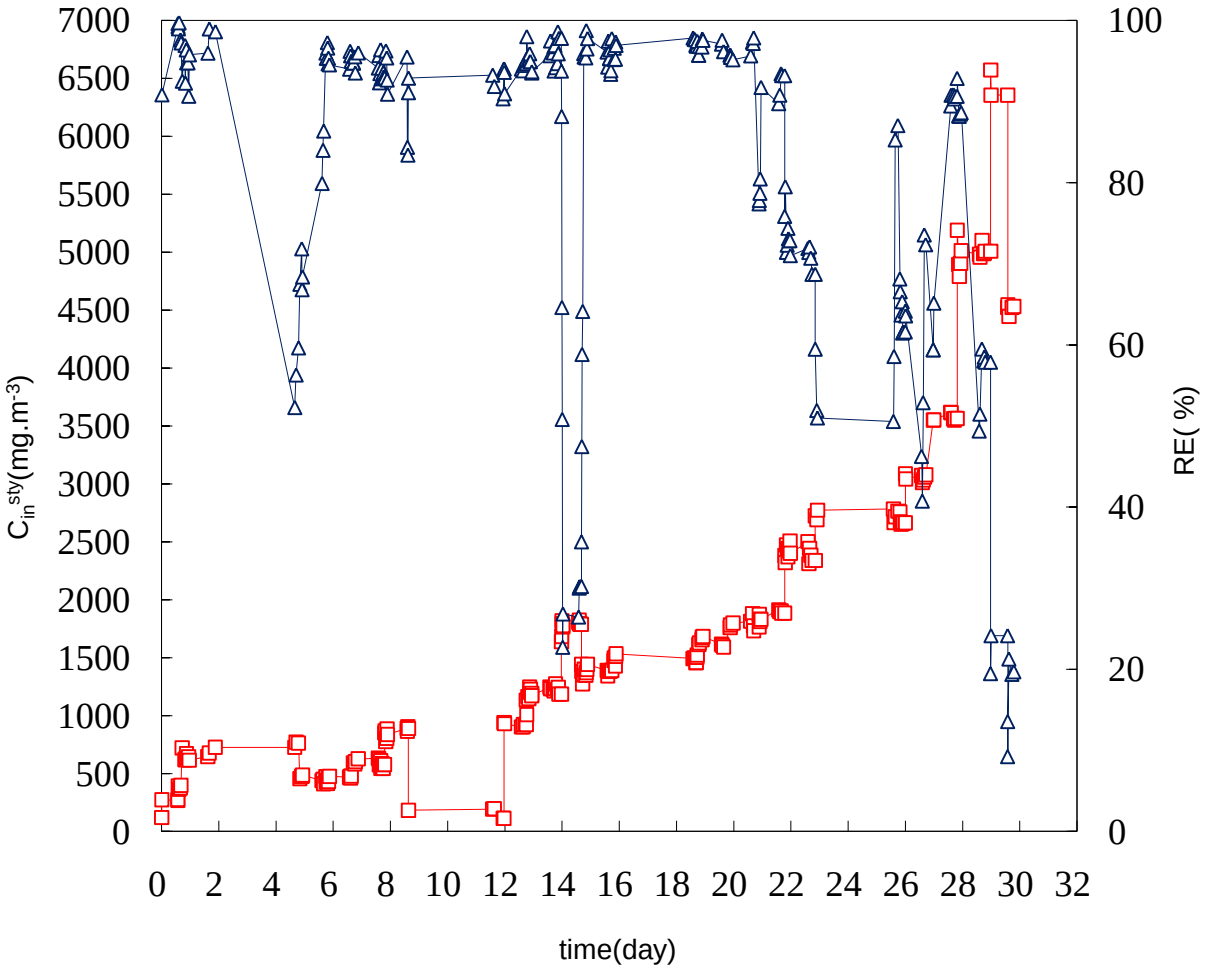


Figure 4-2- Inlet Styrene concentration (C_{in}^{Sty}), □ and Removal efficiency (RE), Δ, for styrene in time (day)

Figure 4-3 represents the variation between the elimination capacity for the styrene (EC^{sty}) and the removal efficiency (RE) in time (days).

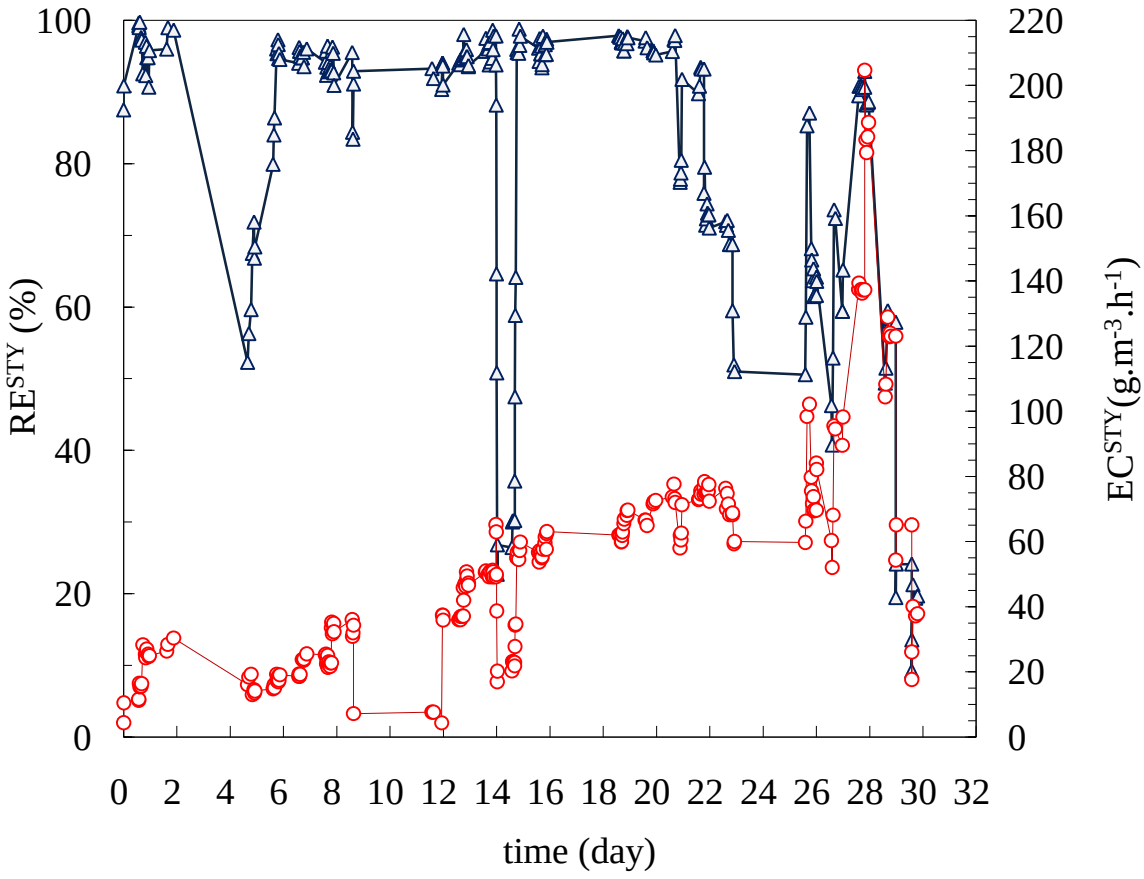


Figure 4-3- Removal efficiency (RE ^{sty}), Δ, elimination capacity (EC ^{sty}), ○, for styrene in time (day)

As showed by equation 2.2, RE^{Sty} is the ratio between the concentration difference (inlet minus outlet) and the inlet styrene concentration; EC ^{sty} only considers the difference between inlet and outlet concentrations, divided by the empty bed volume.

In relation to RE ^{Sty}, at the beginning (days [0-2]), its higher values can be explained by OL ^{Sty} minimum values: as OL ^{Sty} is low, the microorganisms can easily and practically degrade the total styrene, what explains high RE ^{Sty} values. On days [4-5], RE ^{Sty} reaches a low point (≈50%), due to C $_{out}$ ^{Sty} increasing, as it is possible to see in the previous figure. Another important aspect is the fluctuating line between the days [26-28]: this fluctuating line is derived from the fluctuating behavior of C $_{out}$ ^{Sty} shown in figure 4-1: the C $_{out}$ ^{Sty} fluctuation represents a certain instability of the system. On days [28-30] it is observed a great decline of RE ^{Sty} that signifies the weak styrene degradation (minimum ≈20%).

As it is shown, EC ^{sty} has generally the same behavior as RE ^{Sty}, although there are some specific cases where their behaviors lightly diverge, as for example, on days [8-12].

Normally, when C_{in}^{Sty} increases RE^{Sty} increases too (according to figure 4-2), only if C_{out}^{Sty} remain practically constant. This fact can be explained through microorganisms' degradation: the more styrene exists in BSM, the more styrene is degraded by microorganisms. Consequently, RE^{Sty} increases. On the contrary, when styrene degradation starts to decline, it is observed that C_{out}^{Sty} considerably varies, and in these circumstances there is not a constant relation between C_{in}^{Sty} and RE^{Sty} behaviors.

It is essential to interpret the fact that the microorganisms degrade less styrene as time reaches the day thirty. In order to explain that, a study was made between biomass concentration (X_f) values and dissolved oxygen (DOC). These values are represented in figure 4-4.

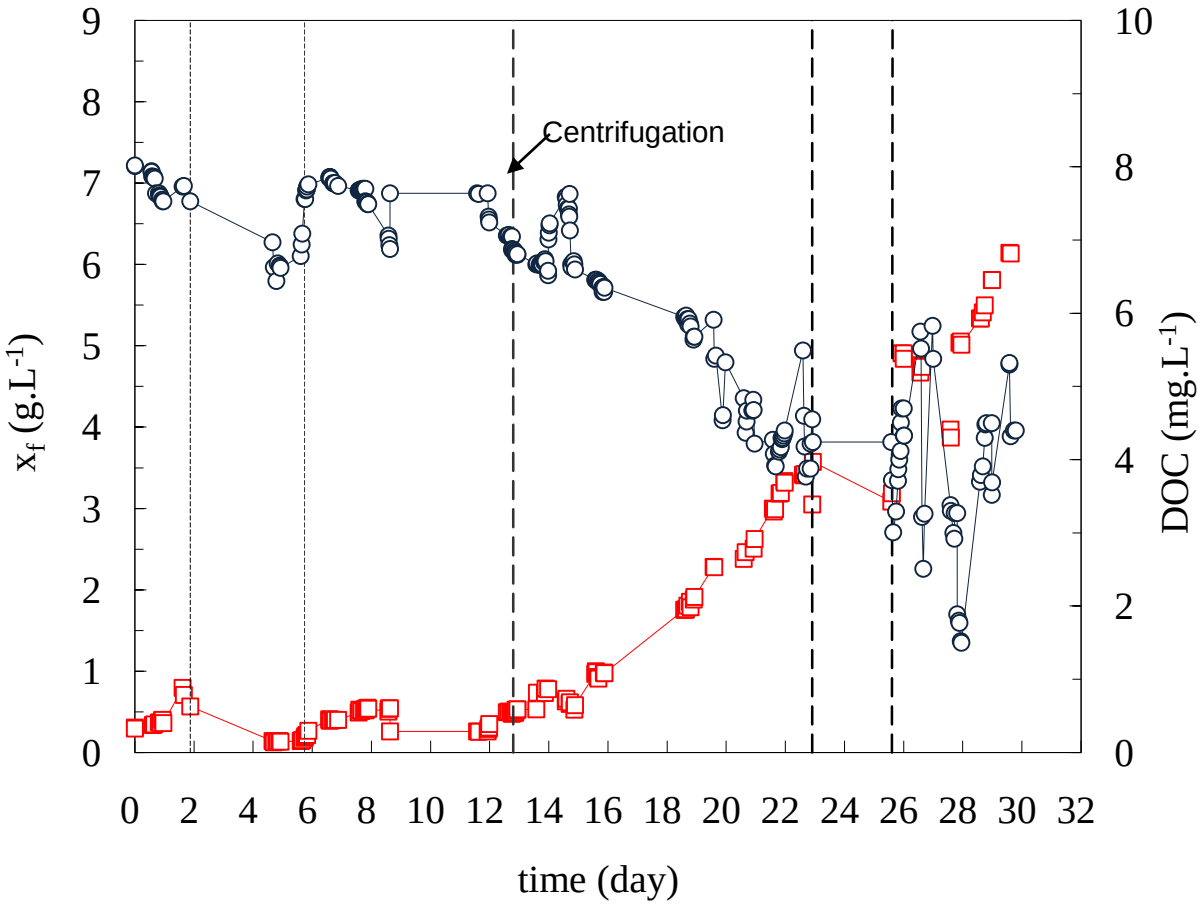


Figure 4-4- Biomass concentration (X_f), □ and dissolved oxygen (DOC)○ along styrene degradation study

It's possible to see an exponential increasing of x_f along time, unlike DOC which has an opposite behaviour.

The vertical dashed lines indicate when the overall suspension volume (BSM) in the BCR was centrifuged; centrifugation has in this experiment an outstanding role: whenever a BSM centrifugation occurs, biomass concentration goes down and, consequently, DOC rises. Biomass concentration decreases, due to solid mass elimination, allowing more contact points between O_2 molecules and microorganisms, and consequently, there´s more O_2 available for the degradation mechanism. Thus, centrifugation is very important to ensure the aerobic nature of the system, so that styrene degradation is not jeopardized along the process.

The minimum DOC value (1.5 $mg.L^{-1}$) is situated between days [28-30], coincident with the maximum C_{in}^{sty} values used.

It is also important to outstand the influence of DOC on C_{out}^{sty} evolution: less O_2 available for the microorganisms prejudices their efficiency, and consecutively, less styrene is degraded and C_{out}^{sty} is increased.

Along the process, biomass has a natural tendency to rise, due to new microorganisms, degradation byproducts…

To conclude, it is possible to say that DOC and X_f are inversely proportional.

In figure 4-5 the variation between the EC_w and RE^{sty} with OL_w is represented.

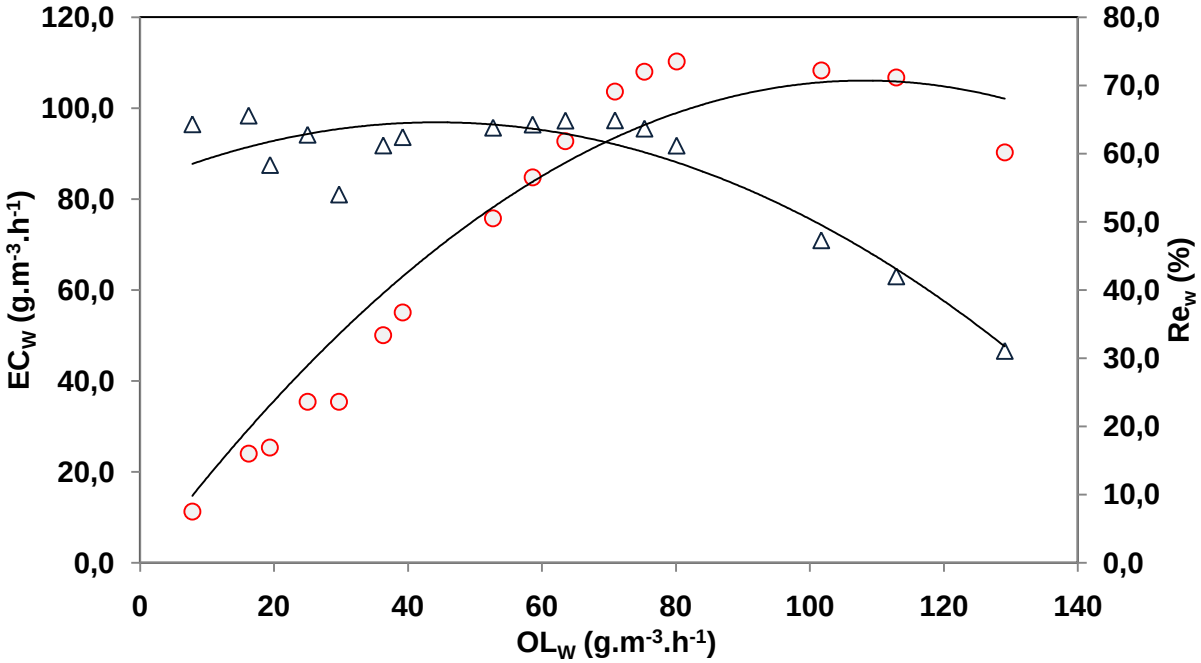


Figure 4-5- Overall elimination capacity (EC_w), ○, and removal efficiency (RE), Δ, related to organic load rate (OL_w) during styrene degradation

At the beginning, one can observe that EC_w and RE_w seem to have different tendencies, what seems to be contradictory, this is, it was supposed, at first impression, that these variables would have the same behavior as they have similar equations and meanings.

In relation to RE_w, its declining is due to OL_w increasing: as organic matter increases, microorganisms have to degrade more and more styrene, and besides that, O_2 available is even less, evolving microorganisms to their saturation state; therefore, microorganisms´ efficiency decreases, C_{out}^{sty} naturally increases, and in this way, RE_w decreases (consult equation 2.2).

$$RE(\%) = \frac{C_{in} - C_{out}}{C_{in}} \times 100 \qquad (2.2)$$

Till approximately 80 $g.m^{-3}.h^{-1}$ of OL_w the RE_w is practically stable (RE≈100%); from that OL_w value the RE decrease significantly till 20%.

Unlike RE_w, EC_w is improved with OL_w increasing and this fact can be explained by C_{in}^{sty} and C_{out}^{sty} different increasing rates: although C_{out}^{sty} is raised with OL_w (as it was explained above), C_{in}^{sty} can increase even more in same conditions, inducing an each more difference between their concentrations, and consecutively, inducing also an EC_w increasing (consult equation 2.3).

$$EC(g.m^{-3}) = \frac{V_f \times (C_{in} - C_{out})}{1000 \times H} \qquad (2.3)$$

Till approximately OL_w 80 $g.m^{-3}.h^{-1}$ the EC_w increased rapidly till approximately100 $g.m^{-3}.h^{-}1$; from that OL_w value the EC_w increased slowly and then remained stable (EC_w= 120 $g.m^{-3}.h^{-1}$).

The principal conclusions to be highlighted are: the maximum values for C_{in}^{sty} and OL^{Sty} are 6500 $mg.m^{-3.}$ and 280 $g.m^{-3.}h^{-1}$ at 28 $^{\underline{th}}$ day. So it is possible to say, after this day the microorganisms lost the pollutant degradation capacity. After that day, the RE and elimination capacity (EC) began to decline as a result of a weak degradation, figure 4-3. In figure 4-4, the biomass concentration (X_f) increase provoked dissolved oxygen (DOC) decline in the basal salt medium (BSM) and consequently, the microorganisms decreased their degradation capacity.

4.1.2 Styrene and acetone degradation in the bubble column reactor (Part II)

In Part II, mixtures of air, styrene and acetone were used as the gaseous stream fed to the BCR. The aim was to study the degradation of both pollutants. As already referred, at the end of Part I, the BCR was “cleaned” by bubbling air till C_{out}^{sty} came to zero. As the biomass used in Part II was exactly the same as in Part I and the microorganisms were already adapted to styrene degradation, it was decided to use higher concentrations of styrene than of acetone. In this way, with a greater loading of styrene the microorganisms’ safety and efficiency were better ensured.

Instead of thirty days of experiment as in Part I, this set of experiments lasted only ten days. This was due to the fact that in the last days efficiency values were exceeding a specific inferior value-limit, in which microorganisms considerably begin to degenerate.

However, the main goal of this experimental part was to find the best reactor´s efficiency value for both pollutants and this was achieved.

Figure 4-5 represents inlet concentrations variation for styrene and acetone during the experimental period of 10 days between day 32 and day 42 as well as the total organic load rate (OLw).

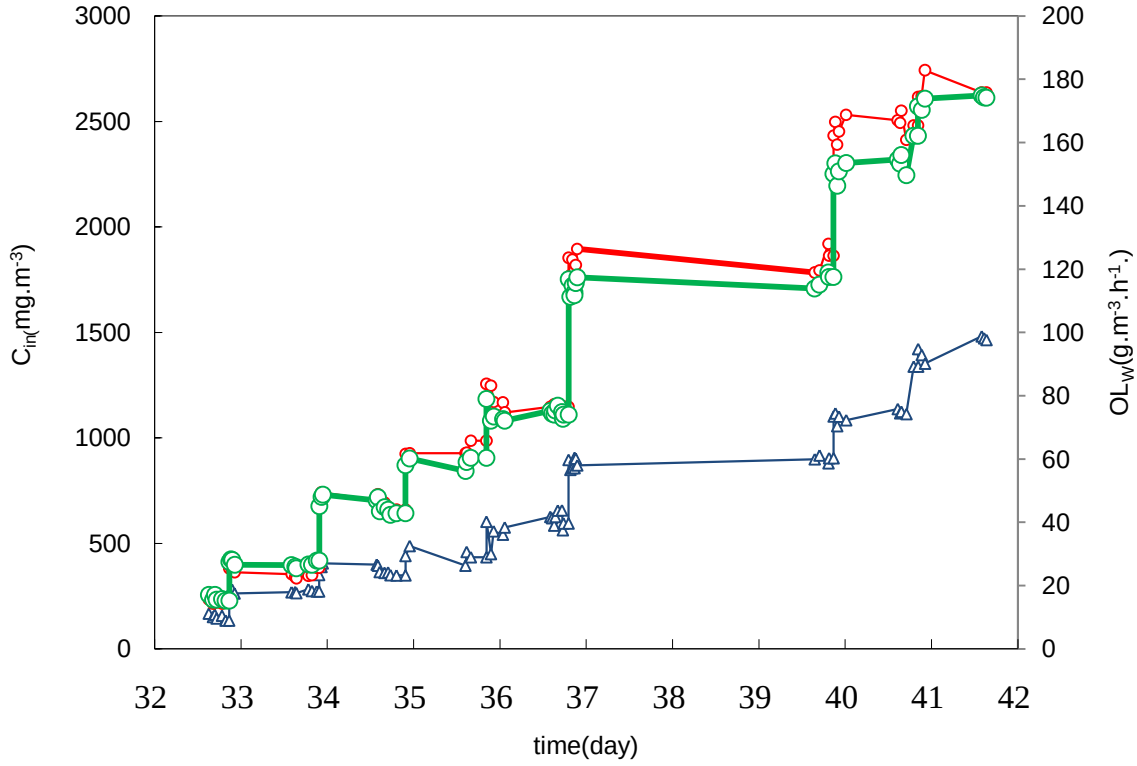


Figure 4-5- Inlet concentration of styrene, ○, and acetone, Δ, and total organic load rate, ○, in time(day) during styrene and acetone degradation period

As was already mentioned before, a larger variation (increasing) on inlet styrene concentration (C_{in}^{Sty}), for a question of microorganisms' security and efficiency.

It is observed also that OL_w has naturally increased in time, as C_{in}^{Sty} and C_{in}^{Ac} are its constituents and have influenced its increment.

The acetone inlet concentration (C_{Ac}^{in}) varied between [153-1389] mg.m^{-3} and styrene inlet concentration (C_{Sty}^{in}) between [225-2659] mg.m^{-3}, OL_w shows the quantity of substrate in the liquid that was in the reactor. So if the C_{in} increases the OL_w (g.m^{-3}.h^{-1}) increases in the same ratio. According with figure 4-5, the OL_w (g.m^{-3}.h^{-1}) varied between [10-180] g.m^{-3}h^{-1}.

The figure 4-7, shows the outlet concentrations variation with total organic load (OL_W) for the air mixture with styrene and acetone.

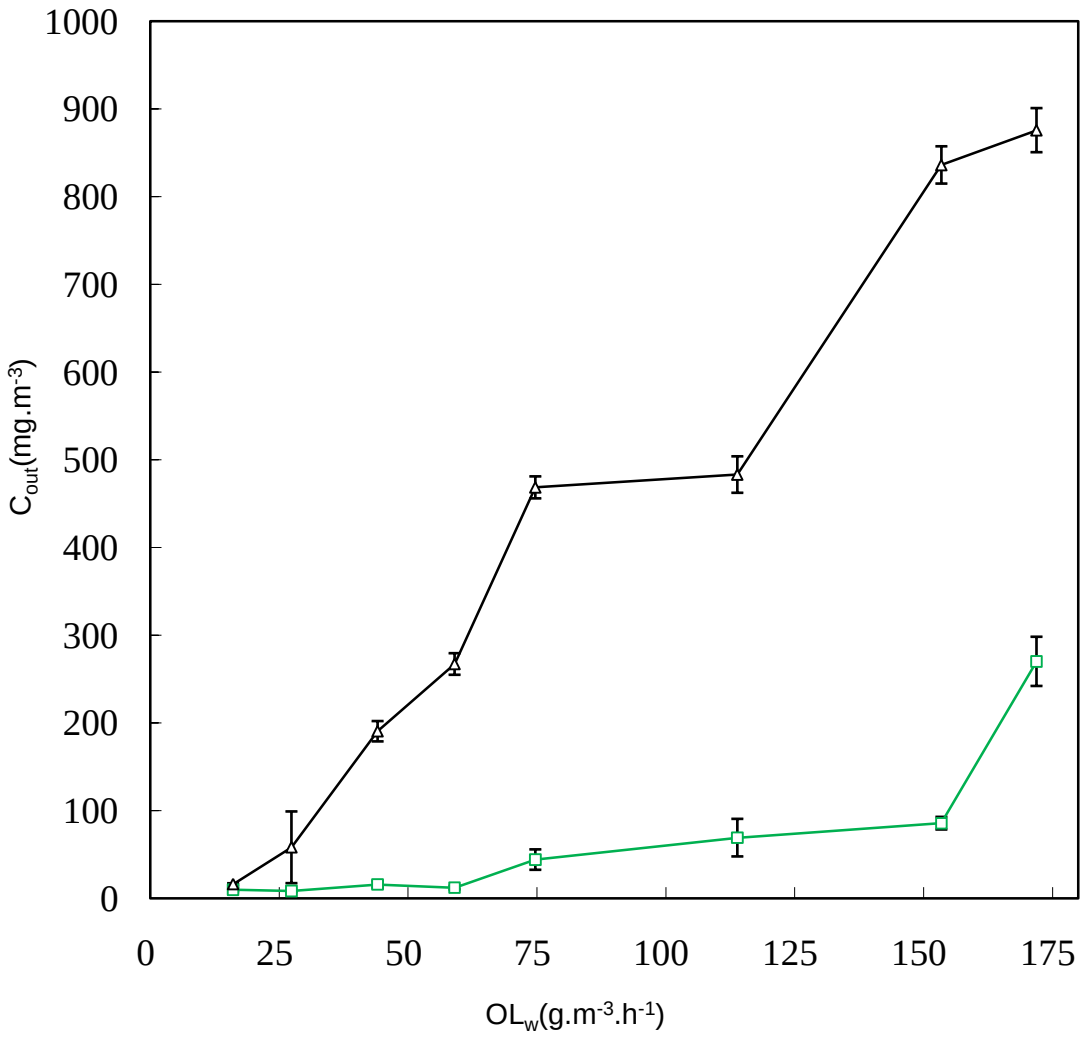


Figure 4-6- Outlet concentrations (C_{out}) variation for styrene, □, and acetone, Δ, with total organic load rate(OL_w).

The outlet styrene concentration is smaller than outlet acetone concentration, what allows to deduce that the microorganisms used degrade styrene better.

From figure 4-6 it may be seen that C^{out} increases with OL_w (consult equation 4.1), for both pollutants. However this behavior is more accentuated for acetone than for styrene. The outlet styrene concentration is smaller than outlet acetone concentration along the experience, what is possible to deduce the microorganisms degraded better the styrene.

Furthermore, it is observed more styrene degradation, in spite of larger C_{in}^{sty} increasing in relation to C_{in}^{Ac} (check previous figure); it is obvious to conclude that microorganisms prefer styrene to their function.

$$OL_w(g.m^{-3}.h^{-1}) = OL^{Sty} + OL^{Ac} = \frac{V_f \times C_{in}{}^{Sty}}{1000 \times V} + \frac{V_f \times C_{in}{}^{Ac}}{1000 \times V} \quad \text{..... (4.1)}$$

In figure 4-7 (A and B) the parameters 4-7 (A and B) the parameters RE and EC are plotted against OL_w, for each VOC.

In figure 4-7 the combined values of RE and EC and OL_w, RE_W and EC_W are analyzed. These values were obtained as follows:

$$OL_w(g.m^{-3}.h^{-1}) = OL^{Sty} + OL^{Ac} = \frac{V_f \times C_{in}{}^{Sty}}{1000 \times V} + \frac{V_f \times C_{in}{}^{Ac}}{1000 \times V} \quad (4.1)$$

$$RE_W(\%) = RE^{Sty} + RE^{Ac} = \frac{C_{in}{}^{Sty} - C_{out}{}^{Sty}}{C_{in}{}^{Sty}} \times 100 + \frac{C_{in}{}^{Ac} - C_{out}{}^{Ac}}{C_{in}{}^{Ac}} \quad (4.2)$$

$$EC_W(g.m^{-3}) = EC^{Sty} + EC_{Ac} = \frac{V_f \times (C_{in}{}^{Sty} - C_{out}{}^{Sty})}{1000 \times V} + \frac{V_f \times (C_{in}{}^{Ac} - C_{out}{}^{Ac})}{1000 \times V} \quad (4.3)$$

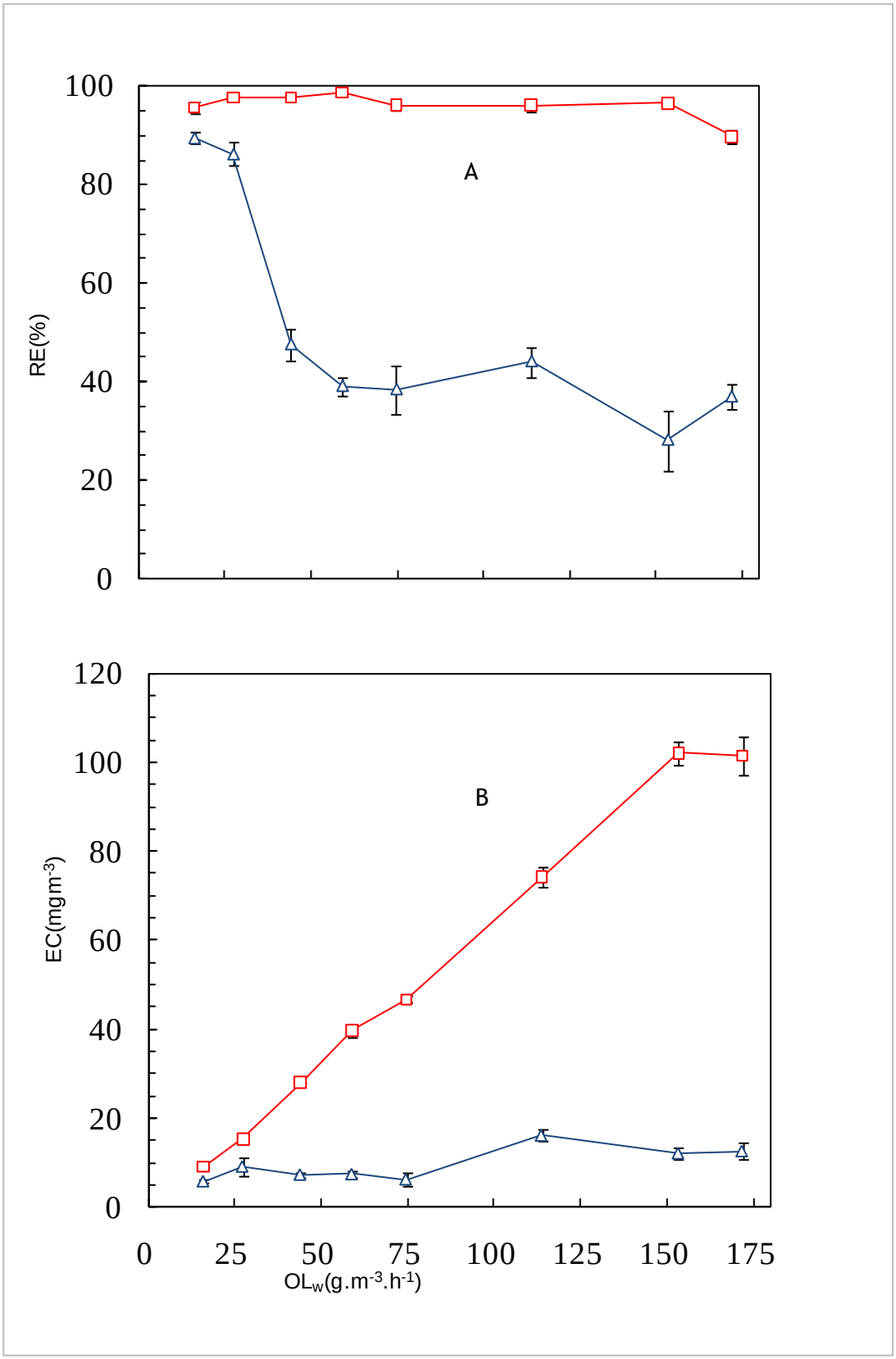


Figure 4-7- Performance characteristics: A- □- RE^{sty}, Δ- RE^{Ac}; B- □-EC^{Sty}, Δ — EC^{Ac}

Observed figure 4-7, it is possible to conclude the same than figure 4-6. The microorganisms degraded better styrene than acetone: RE^{Sty} is larger than RE^{Ac} along OL_w increasing and EC^{Sty} is greater than EC^{Ac}(C_{out}^{Ac} bigger than C_{out}^{Sty} - check previous figure).

It is curious to see that RE^{sty} was continuous very high (almost one hundred per cent) along OL_w increasing, expressing that not only C_{in}^{Sty} rapidly increases but also C_{out}^{Sty} is very low; RE^{sty} was not affected by OL_w increasing. On the contrary, OL_w increasing affected RE^{Ac}.

At last, it is obtained C_{in}^{Ac} on which RE^{Ac} was maximum: this maximum (≈40%) corresponds to OL_w value of, approximately, 50 $g.m^{-3}.h^{-1}$. From this value is possible to obtain the correspondent C_{in}^{Ac} of 750 $mg.m^{-3}$(see figure 4-5).

In figure 4-7A, RE^{sty} is practically a hundred per cent, due to a very low C_{out}^{Sty} compared to C_{in}^{Sty}: as OL_w is increased, the microorganisms degraded effectively the styrene;

In the next figure is possible to evaluate the distribution of overall removal capacity (RE_W), total elimination capacity *(*EC_W*)* with total organic load *(*OL_W).

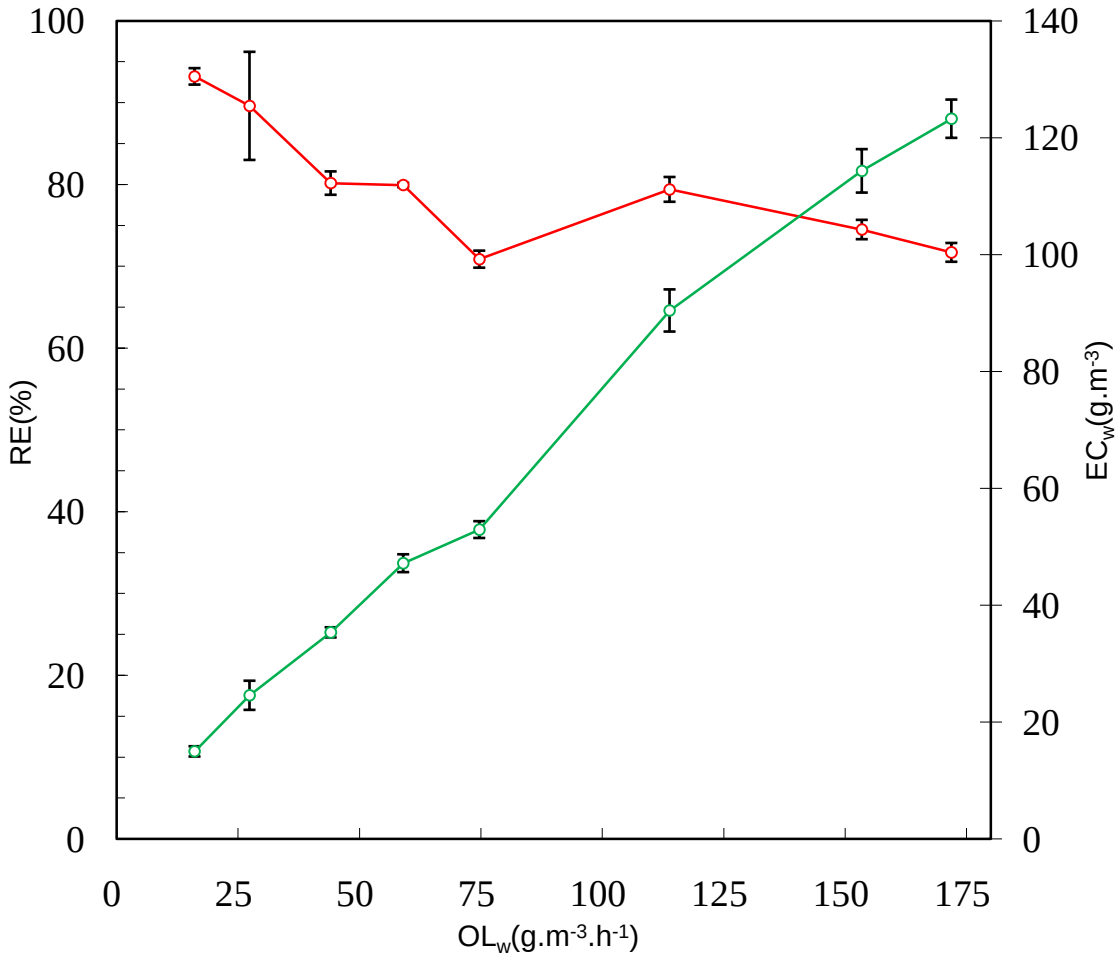


Figure 4-8- Variation of overall removal capacity(RE_w), ○, total elimination capacity (EC_w), ○, with total organic loud (OL_w).

Once more, and confirming previous figure, is observed the efficiency and elimination capacity, in general. RE_w and EC_w evolved differently and oppositely: while EC_w increased, RE_w decreased.

In relation to RE_w, it presents a negative slope, due to RE^{Ac} contribution: as RE^{Ac} has decreased along the process, that influenced negatively RE_w in spite of a very high RE^{sty}; however, RE_w decreasing was not to sharp.

Regarding to $EC_{w,,}$ it presents a positive slope, due to EC^{Sty} contribution: as EC^{Sty} has considerably increased along the process, that influenced positively EC_w; however, EC_w had a lower slope than EC^{Sty}, due to EC^{Ac} contribution.

Summarizing, EC_w and RE_w are inversely proportional.

4.1.3 Loading styrene at Cin Ac= 750 mg.m^{-3} = constant.

In the previous chapter, the best acetone efficiency for a pollutant mixture air-styrene/acetone was the centre of study, in which after knowing the referred efficiency, it was possible to know the correspondent C_{in}^{Ac} (750 mg.m^{-3}).

In this chapter, it was pretended to study the behaviour of the microorganisms biodegradation, using C_{in}^{Ac} =750 mg.m^{-3}=constant and increasing progressively C_{in}^{Sty}.

In figure 4-11 are demonstrated two graphics, representing styrene/acetone inlet concentration and RE^{Sty} and RE^{Ac} along the increase of OL_W, respectively.

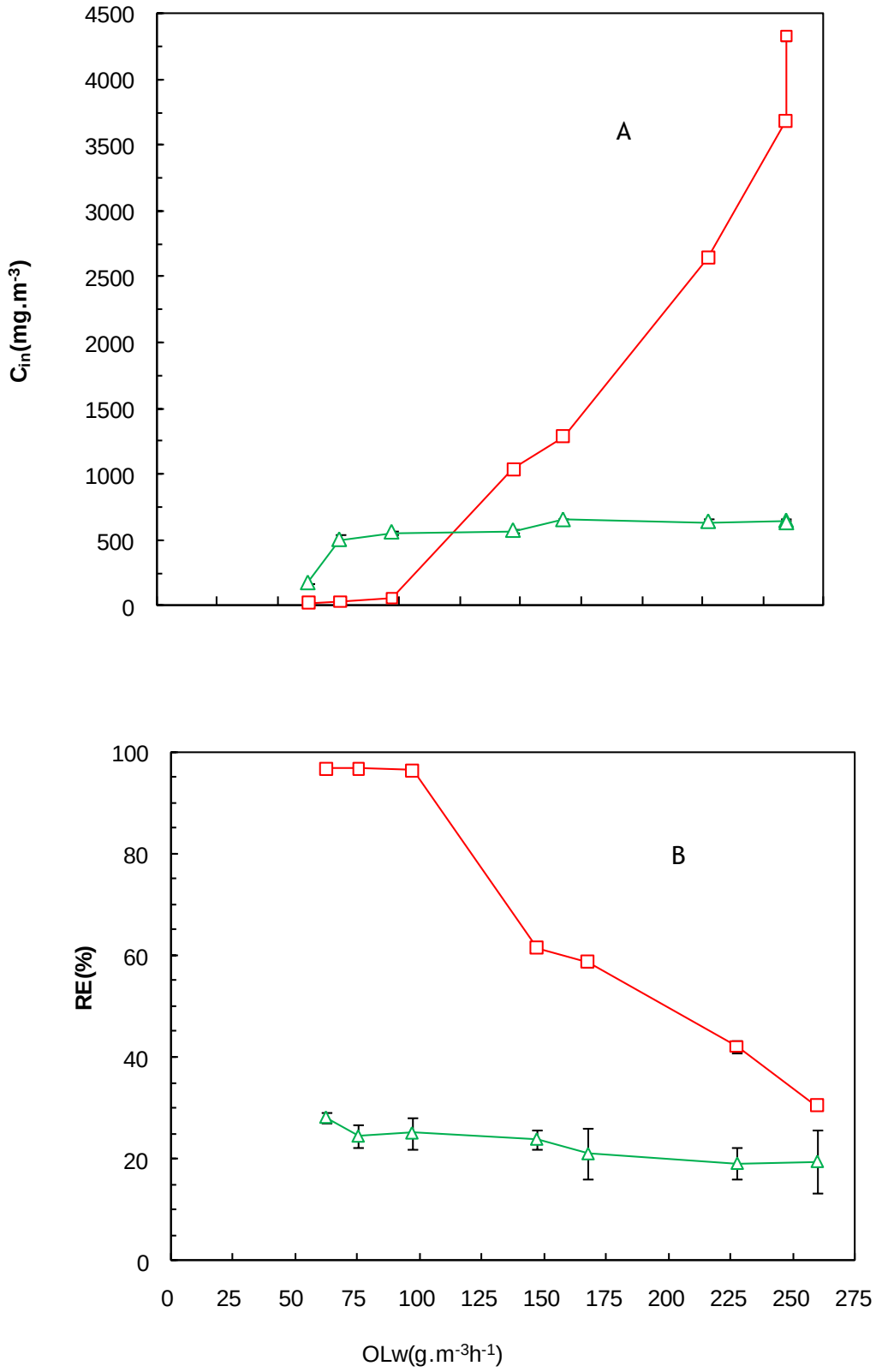


Figure 4-9- Performance characteristics: A- □- C_{in}^{sty}, Δ- C_{in}^{Ac}; B- □-RE ^{Sty}, Δ - RE^{AC}.

RE ^{Sty} decreases with the increasing of OL_W in the liquid of the reactor, this is, the microorganisms lost the capacity of biodegradation along the process, mainly caused by their non-functionality (degeneration).

RE ^{Ac} stays practically constant along the process. This fact was evident, due to constant C_{in}^{Ac}.

The main emphasizing point is the maximum C_{in}^{Sty} (≈4200 mg.m-3) on which the RE^{Sty} minimum (≈30%) is acceptable. The mentioned C_{in}^{Sty} is the maximum value-limit on which the microorganisms can respond and operate.

4.1.4 Biomass vs. dry mass

In this section, it is focused the evolution of biomass and dry mass along time.

In figure 4-11 is represented the relation between dry mass and biomass along all the experience, table II-2..

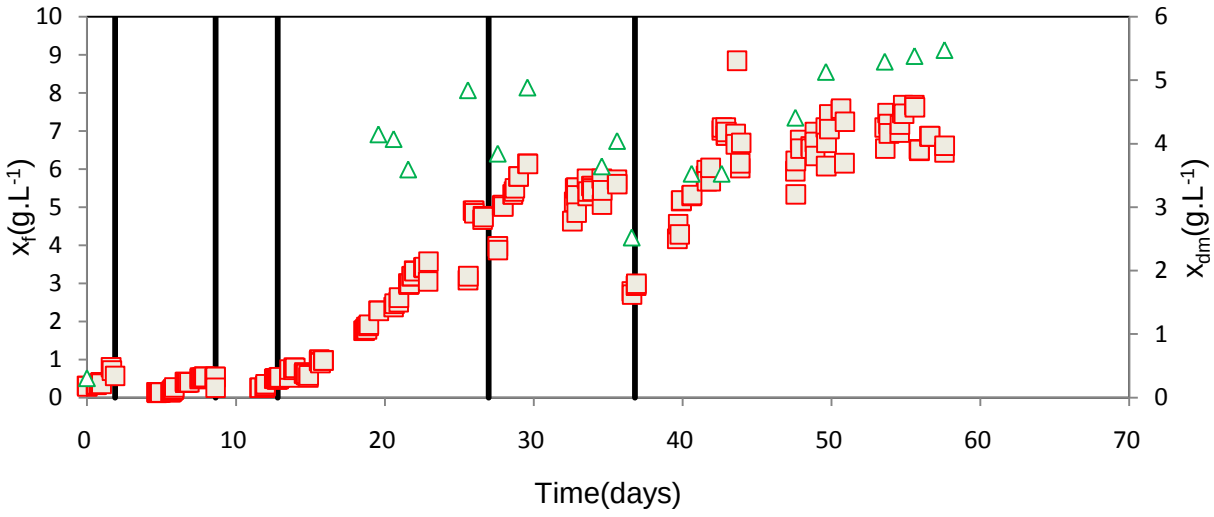


Figure 4-10- Biomass changes during the reactor operation, Δ, and photometric biomass, □.

The vertical lines represent the overall suspension volume that was centrifuged.

X_f and X_{dm} increases gradually during the experience and they seem to share the same behavior.

On each centrifugation, both X_{dm} and X_f generally decreased: centrifugation can cause a reducing of density in the liquid of reactor. Centrifugation was always needed whenever it was considerably difficult for the microorganisms to degrade pollutant gases, and therefore, it was essential that both X_{dm} and X_f could decrease, in order to maintain their functionality

4.1.5 Microbiologic

In this section, it is focused the several microorganisms in the biomass. For this purpose, it were prepared four different cultures (see section 3.3.1.2).

After two days, in all cultures were observed microorganisms colonies. Observing the growing inside the cultures, it was possible to identify these species, namely *pseudomonas* and *eukaryotes*. The microorganisms available in biomass are: *Xanthobacter*, *Enterobacter*, *Nocardia*, *Corynebacterium*, *Rhodococcus* and *Pseudomonas Sp*.

One of four cultures was prepared only with gas (styrene, acetone, styrene/acetone) and without carbon. The gas used was the energy source for microorganisms growing- up inside the culture. In all of these types of cultures appeared several microorganisms colonies, so is possible to conclude the microorganisms were able to degrade the gases mixtures mentioned above.

5 Conclusions

The experience was divided in three parts, so the conclusions are divided in the same way.

The first part is related only to styrene pollutant degradation. According to figure 4-1, it has occurred an exponential increasing of styrene inlet concentration (C_{in}^{sty}) and styrene organic load (OL^{sty}); they varied between 250 to 6500 $mg.m^{-3.}$ and 0 to 280 $g.m^{-3.}h^{-1}$, respectively. The maximum values are 6500 $mg.m^{-3.}$ and 280 $g.m^{-3.}h^{-1}$ at 28 th day. So it is possible to say, after this day the microorganisms lost the pollutant degradation capacity, in other words, the styrene outlet concentration(C_{out}^{sty}) increased abruptly. In figure 4-3, the removal efficiency (RE) was practically maintained 100%, although it had some minimum values according to C_{out}^{sty} increasing. This means that there was excellent pollutant degradation by the microorganisms, despite the C_{in}^{sty} exponential increasing. After 28 th day, the RE and elimination capacity (EC) began to decline as a result of a weak degradation, figure 4-3. In figure 4-4, the biomass concentration (X_f) increasing provoked dissolved oxygen (DOC) decline in the basal salt medium (BSM) and consequently, the microorganisms decreased their degradation capacity. Furthermore, the system had a tendency of becoming anaerobiotic. The centrifugations represented by vertical dashed lines, interfered on the system, decreasing the X_f and increasing the DOC quantities. In figure 4-5, the RE decreased as a result of C_{out}^{sty} increasing, due also to overall organic loading rate (OL_W) increasing. The overall elimination capacity (EC_W) increasing is related to different raising ratios: C_{in}^{sty} increasing (manipulated) had been superior to C_{out}^{sty} and their difference had been increased along the experience.

The second part is related to a mixture of gas streams degradation, namely styrene, acetone and air. In figure 4-6 OL_w increased along with the mixture inlet concentration (C_{in}.) increasing, in other words, the styrene and acetone injection interfered on the OL_w quantity in the BSM. In figures 4-7 and 4-8, it is possible to conclude that microorganisms degraded better the styrene than acetone: in figure 4-7 it is shown that C_{out}^{sty} is always inferior than acetone outlet concentration (C_{out}^{Ac}); in figure 4-8 styrene removal efficiency (RE^{Sty}) and the styrene elimination capacity (EC^{Sty}) are always superior than acetone removal efficiency (RE^{Ac}) and the acetone elimination capacity (EC^{Ac}) in course of the experiment. In figure 4-8A, it is clear that the microorganisms degradation of styrene is highly efficient (RE≈100%), while in figure 4-8B, it is obvious that microorganisms degradation of acetone is lowly efficient ($EC\approx20mg\text{-}m^{-3}$).

At last, it is obtained C_{in}^{Ac} on which RE^{Ac} was maximum: this maximum (≈40%) corresponds to OL_w value of, approximately, 50 $g.m^{-3}.h^{-1}$. From this value is possible to obtain the correspondent C_{in}^{Ac} of 750 $mg.m^{-3}$(see figure 4-6).

The third part is related to a mixture of gases streams degradation, styrene, acetone and air, using a constant acetone inlet concentration (C_{in}^{Ac}) equal to 750mg.m^{-3} in which the acetone removal efficiency (RE^{Ac}) was maximum. In figure 4-10 it is possible to conclude that microorganisms could degrade a higher C_{in}^{sty} than in the previous part. The constant C_{in}^{Ac} has improved indeed the microorganisms' degradation capacity in the presence of high quantity of the pollutant and amplified their performance capacity.

At the end of the experimental work, it is studied the microorganisms presented in the biomass. They were namely: *Xanthobacter*, *Enterobacter*, *Nocardia*, *Corynebacterium*, *Rhodococcus* and *Pseudomonas Sp*

6 References

[1]- Carvalho M.F., Duque A.F., Moura S.C., Amorim C.L., Jorge Ferreira R.M., Castro P.M.L., " Biological treatment of a contaminated gaseous emission from a leather industry in a suspended- growth bioreactor", Chemosphere 74, 232-238 (2009)

[2]- Burgess Joanna E., Parsons Simon A., Stuetz Richard M., "Developments in odour control and waste gas treatment biotechnology: a review", Biotechnology Advances 19, 35-63 (2001).

[3]- Hájková Dagmar,"Degradace tekavých polutantu z odpadních plynu v biofiltrech"Diplomová práce, Vysoká skola chemicko- technologická v Praze, Ústav kvasné chemie a bioinzénýrství, 2002/2003.

[4]-http://www.environment-agency.gov.uk/business/topics/pollution/232.aspx (appointment: 18/8/09)

[5]- http://www.epa.gov/chemfact/styre-sd.txt (appointment: 18/8/9)

[6]- Shimada Kunio, Kimura Eiji, Yasui Yohko, Tanaka Hideki et all.;" Styrene formation by the decomposition by Pichia Carsonii of trans- cinnamic acid added a grand fish product" applied and environmental microbiology; 1577- 1582 (1992).

[7] http://upload.wikimedia.org/wikipedia/commons/c/c5/Styrene.svg (appointment: 10/06/09).

[8]- Jorio Hasnaa, Bibeau Louise, Heitz Michele;" Biofiltration of air contamined by styrene: effect of nitrogen supply, gas flower rate, and inlet concentration", Environ.Sci.Technol.34, 1764-1771(2000).

[9] - http://www.epa.gov/chemfact/styre-sd.txt (appointment: 18/8/9)

[10]- http://www.atsdr.cdc.gov/toxprofiles/tp21-c3.pdf (appointment: 18/8/9)

[11]-http://www.environmentwriter.org/resources/backissues/chemicals/acetone.htm (appointment: 18/8/9)

[12]- http://www.bohnbiofilter.com/html/what_is_biofiltration_.html (appointment: 16/06/09)

[13]- http://alexandria.tue.nl/repository/freearticles/620777.pdf (appointment: 20/8/9)

[14]- Jang J.H., Hirai M., Shoda M.; " Styrene degradation by Pseudomonas Sp. SR-5 in biofilters with organic and inorganic packing materials"; Appl Microbiol Biotechnol 65: 349-355(2004).

[15]- Deshusses Marc A, "Biological waste air treatment in biofilters", Current Opinion in Biotechnology, 8: 335-339 (1997).

[16]-http://128.113.2.9/dept/chem-eng/Biotech-Environ/MISC/biofilt/biofiltration.htm (appointment: 19/8/9)

[17]- Lens Piet N.L., Kennes Christian, Cloirec Pierre Le; " Waste gases treatment for resource recovery"; IWA publishing, 250- 252 (2006).

[18]- http://www.p2pays.org/ref/37/36915.pdf (appointment: 19/8/9)

[19]- VDI(ed.): VDI- Richtiline 3477: Biologische Abluftreinigung, Biofilter, Dusseldorf,1985.

[20]- Breihofer, D.; Mielenz, A., Rentz, O. :Maβnahmen zur Minderung der Emissionen von SO2, NOx und VOC bei stationaren Quellen in der Bundersrepublik Deutschland, Karlsruhe, May 1991.

[21]- Kennes Christian, Thalasso Fréderic, “Waste gas biotreatment technology”, J. Chem. Technol, Biotechnol, 72, 303-319 (1998).

[22] - Ramirez Antonio Avalos, Jones J. Peter, Heitz Michéle; “Control of methanol vapours in a biotrickling filter: performance analysis and experimental determination of partition coefficient”; Bioresource Technology 100, 1573- 1581 (2009).

[23] - Velasco Ana, Alonso Sergio, Gracia José L., Pereira J., Diaz Eduardo; “Genetic and functional analysis of the styrene catabolic cluster of *Pseudomonas Sp. Strain Y2*”; 180 (5); 1063- 1071 (1998).

[24]- Itok Nobuya, Morihana Ritsuko et all; “Purification and characterization of *Phenylacetaldehyde reductase* from a styrene- assimilating *Corynebacterium Strain*-ST10”; Applied and environmental microbiology; 3783-3788; (1997).

[25]- Shimada Kunio, Kimura Eiji, Yasui Yohko, Tanaka Hideki et all.;” Styrene formation by the decomposition by Pichia Carsonii of trans- cinnamic acid added a grand fish product” applied and environmental microbiology; 1577- 1582 (1992).

[26]- O’ Leary Niall, O´Connor Kevin E., Dobson Alan D.W.; “Biochemistry genetics and physiology of microbial styrene degradation”; FEMS Microbiology reviews 26; 403- 417 (2002).

[27]- O’ Leary Niall D., O’ Connor Kevin E., Duetz Wouter, Dobson Alan D.W.; “Transcriptional regulation of styrene degradation in *Pseudomonas putida CA-3*”; Microbiology; 147; 973-979 (2001).

[28] - Ensign Scott A., Small Frederick J., Allen Jeffrey R., Sluis Miriam K.; “New roles for CO_2 in the microbial metabolism of aliphatic epoxides and ketones”; Arch microbial 169; 179-187 (1998).

[29]- Sluis Miriam K., Small Frederick J., Allen Jeffrey R., Ensign Scott A.; “Involvement of an ATP- dependent carboxylase in a CO_2- Dependent pathway of acetone metabolism by *Xanthobacter strain PY2*”; Journal of Bacteriology, 4020-4026 (1996).

[30]- Platen herald, Schink Bernhard; “Enzymes involved in anaerobic degradation of acetone by a denitrifying bacterium”; Biodegradation 1; 243-251 (1990).

[31]- Janssen Peter H., Schink Bernhard; “Catabolic and anabolic enzyme activities and energetics of acetone metabolism”; Journal of Bacteriology; 277- 282 (1995).

[32]-http://128.113.2.9/dept/chem-eng/Biotech-Environ/MISC/biofilt/biofiltration.htm (appointment: 19/8/9)

[33]-www.unioeste.br/cursos/cascavel/biotecnologia/aulas/2008/biofiltra%C3%A7%C3%A3o.pdf (appointment: 20/8/9)

[34] - http://alexandria.tue.nl/repository/freearticles/620777.pdf (appointment: 20/8/9)

I. Annexes *I*

I.1 Examples of calculation

The empty bed residence time (EBRT) was maintained 84.8 sec, i.e., the V_g = 1L/min. In order to determine the parameters was necessary some considerations:

- V_f= 13.6 $m.h^{-1}$
- H= 0.32 m

I.1.1 Determination of the degradation of pollutant mixture air- styrene in a bubble column reactor

The calculus of parameters was made by the average of the experimental results. In this example are used the C_{in}^{Sty} = 191$mg.m^{-3}$, C_{out}^{Sty} =14 $mg.m^{-3}$ and time of inoculation [8-11] days.

$$OL^{Sty} = \frac{V_f \times C_{in}}{H \times 1000} = \frac{13.6 \times 191}{1000 \times 0.32} = 7.8\ g.m^{-3}.h^{-1}$$

$$RE^{Sty} = \frac{C_{in} - C_{out}}{C_{in}} \times 100 = \frac{191 - 14}{191} \times 100 = 96.48\%$$

$$EC^{Sty} = \frac{V_f (C_{in} - C_{out})}{H} = \frac{13.6(191 - 14)}{1000 \times 0.32} = 7.52\, g.m^{-3}.h^{-1}$$

I.1.2 Calculus of the mixture air + styrene+ acetone degradation in a bubble column reactor (BCR)

The determination of parameters was calculated from the average of the experimental results. In this example are used the C_{in}^{Sty} = 255mg.m^{-3}, C_{in}^{Ac} = 153mg.m^{-3}, C_{out}^{Sty} =9.7 mg.m^{-3}, C_{out}^{Ac} = 16mg.m^{-3} and time of inoculation [30-32] days.

This example is first calculated only for the mixture air+ styrene, then, it is calculated for the mixture air+ acetone and finally is calculated the two mixtures.

- ✓ Pollutant mixture air+ styrene

$$OL^{Sty} = \frac{V_f \times C_{in}}{1000 \times H} = \frac{13.6 \times 255}{1000 \times 0.32} = 9.1\ g.m^{-3}.h^{-1}$$

$$RE^{Sty} = \frac{C_{in} - C_{out}}{C_{in}} \times 100 = \frac{255 - 9.7}{255} \times 100 = 95.7\%$$

$$EC^{Sty} = \frac{V_f (C_{in} - C_{out})}{1000 \times H} = \frac{13.6(255 - 9.7)}{1000 \times 0.32} = 9.1 g.m^{-3}.h^{-1}$$

- ✓ Pollutant mixture air+ acetone

$$OL^{Ac} = \frac{V_f \times C_{in}}{1000 \times H} = \frac{13.6 \times 153}{1000 \times 0.32} = 7.0\ g.m^{-3}.h^{-1}$$

$$RE^{Ac} = \frac{C_{in} - C_{out}}{C_{in}} \times 100 = \frac{153 - 16.0}{153} \times 100 = 89.5\%$$

$$EC^{Ac} = \frac{V_f (C_{in} - C_{out})}{1000 \times H} = \frac{13.6(153 - 16.0)}{1000 \times 0.32} = 5.8 g.m^{-3}.h^{-1}$$

✓ Pollutant mixture air+ styrene+ acetone

$$OL_W = OL^{Sty} + OL^{Ac} = 9.1 + 7.0 = 16.5\ g.m^{-3}.h^{-1}$$

$$EC_W = EC^{St}y + Ec^{Ac} = 9.1 + 5.8 = 15.0 g.m^{-3}.h^{-1}$$

$$RE_W = \frac{EC_w}{Ol_w} = \frac{15.0}{16.1} \times 100 = 93.2\%$$

I.1.3 Calculus of the degradation of mixture air+ styrene+ acetone in a bubble column reactor (BCR), using the C_{in}^{Ac}= 750 mg.m^{-3} = constant.

The results are calculated in the same manner as in 1.1.2. The only difference is that the C_{in}^{Ac} is constant and equal to 750mg.min $^{-3}$.

I.2 Experimental results

I.2.1 Overview

I.2.1.1 Styrene degradation in the bubble column reactor

In the next table are represented the experimental results for the Part I of the experimental work, pollutant mixture air+ styrene. The experience was realized in BCR, with the following parameters:

- EBRT=84.8 sec
- V_g= 1 L/min.
- V_f= 13.6 mg.m^{-3}

Table I-1- Experimental results of styrene degrade in bubble column reactor (BCR)

Time after inoculation (day)	C_{in}^{Sty} *(mg. m^{-3})*	C_{out}^{Sty} *(mg. m^{-3})*
8-11	191	14.0
1	382	6.25
4-6	455	58.0
6-7	589	34.0
1-4	695	140.0
7-8	856	71.0
11-12	922	59.0
12-13	1242	53.0
14-15	1380	49.0
15-18	1496	41.0
18-19	1671	44.0
19-20	1775	80.0
13-14	1886	155.0
21-22	2396	695.0
25-26	2659	984.0
26-27	3039	1622.0

Afterwards, are represented the overall calculated results of the mixture air+ styrene pollutant (table I-2). The results are calculated from the average of overall experimental results. In the section I.1.1 is represented the calculated examples for the parameters in the next table.

Table I-2 Calculated results for styrene degradation in bubble column reactor.

Time of operation (day)	OL_W ($g.m^{-3}.h^{-1}$)	RE^{Sty} (%)	EC^{Sty} ($g.m^{-3}.h^{-1}$)
8-11	7.8	96.5	7.5
1	16.2	15.6	98.4
4-6	19.4	16.9	87.5
6-7	25.0	23.6	94.2
1-4	29.7	23.6	81.0
7-8	36.3	33.4	91.8
11-12	39.2	36.7	93.7
12-13	52.7	50.5	95.7
14-15	58.6	56.5	96.4
15-18	63.5	61.8	97.3
18-19	70.9	69.1	97.3
19-20	75.3	72.0	95.5
13-14	80.1	73.5	91.8
21-22	101.7	72.2	71.0
25-26	112.9	71.2	63.0
26-27	129.1	60.2	46.6

I.2.1.2 Degradation of mixture air+ styrene+ acetone in a bubble column reactor (BCR)

In the table I-3, is possible to see the results of Part II. The experience was made in the bubble column reactor (BCR), being used the same parameters as the part before:

- EBRT=84.8 sec
- V_g= 1 L/min.
- V_f= 13.6 mg.m^{-3}

Table I-3 - Experimental results for the pollutant styrene and acetone

Time after inoculation (day)	$C^{in}\frac{Sty}{Ac}$ *(mg.m^{-3})*	$C^{out}\frac{Sty}{Ac}$ *(mg.m^{-3})*
30-32	$\frac{225}{153}$	$\frac{9.7}{16.0}$
32-33	$\frac{371}{274}$	$\frac{8.3}{58.0}$
33-34	$\frac{675}{363}$	$\frac{15.7}{190.3}$
34-35	$\frac{948}{442}$	$\frac{12.0}{267.0}$
35-36	$\frac{1143}{616}$	$\frac{44.0}{468.4}$
36-39	$\frac{1817}{865}$	$\frac{69.0}{483.0}$
39-40	$\frac{2491}{1123}$	$\frac{85.5}{836.0}$
40-41	$\frac{2659}{1389}$	$\frac{270.0}{875.7}$

In the table I-4, are represented the calculated results of the pollutant mixture air+ styrene+ acetone. The results are calculated from the average of the overall experimental results in this part. In the section I.1.2 are represented the calculated examples for the parameters in the next table.

Table I-4- Calculated results for the pollutant styrene and acetone

Time after inoculation (day)	*OL* $\frac{Sty}{Ac}$ *($g.m^{-3}.h^{-1}$)*	*OL_W ($g.m^{-3}.h^{-1}$)*	*RE* $\frac{Sty}{Ac}$ *(%)*	*RE_W (%)*	*EC* $\frac{Sty}{Ac}$ *($g.m^{-3}.h^{-1}$)*	*EC_W ($g.m^{-3}.h^{-1}$)*
30-32	$\frac{9.4}{7.0}$	16.1	$\frac{95.7}{89.5}$	93.2	$\frac{9.1}{5.8}$	15.0
32-33	$\frac{15.8}{12.0}$	27.4	$\frac{97.8}{86.3}$	89.6	$\frac{15.4}{9.2}$	24.6
33-34	$\frac{28.7}{15.0}$	44.1	$\frac{97.7}{47.6}$	80.2	$\frac{28.0}{7.3}$	35.3
34-35	$\frac{40.2}{19.0}$	59.0	$\frac{98.7}{39.1}$	79.9	$\frac{39.7}{7.4}$	47.2
35-36	$\frac{48.5}{26.0}$	74.7	$\frac{96.2}{38.4}$	70.9	$\frac{46.7}{6.3}$	52.9
36-39	$\frac{77.1}{37.0}$	113.9	$\frac{96.2}{44.1}$	79.9	$\frac{74.2}{16.2}$	90.4
39-40	$\frac{105.8}{48.0}$	153.4	$\frac{96.6}{28.1}$	74.5	$\frac{102.1}{12.2}$	114.3
40-41	$\frac{112.9}{59.0}$	171.9	$\frac{89.8}{36.9}$	71.7	$\frac{101.5}{12.7}$	123.2

I.2.1.3 Bubble column reactor- loading by C_{in}^{Sty} keeping C_{in}^{Ac} =750 mg.m^{-3} (Part III).

In table I-5 are represented the experimental results of mixture air+ styrene+ acetone (C_{in}^{Ac}= 750 mg.m^{-3}= constant). The experience was made in the bubble column reactor, with same parameters as the other parts:

- EBRT=84.8 sec
- V_g= 1 L/min.
- V_f= 13.6 mg.m^{-3}

Table I-5- Experimental results for the pollutant styrene and acetone (C_{in}^{Ac}=750 mg.m^{-3}).

Time after inoculation (day)	$C^{in}\frac{Sty}{Ac}$ *(mg.m^{-3})*	$C^{out}\frac{Sty}{Ac}$ *(mg.m^{-3})*
47-48	$\frac{681.0}{793.0}$	$\frac{22.9}{173.1}$
48-49	$\frac{941.0}{837.0}$	$\frac{31.4}{500.7}$
49-50	$\frac{1535.0}{751.0}$	$\frac{57.1}{557.7}$
50-53	$\frac{2687.0}{775.0}$	$\frac{1037.3}{570.3}$
53-54	$\frac{3120.0}{828.0}$	$\frac{1286.2}{651.3}$
54-55	$\frac{4565.0}{789.0}$	$\frac{2645.7}{637.5}$
56-56	$\frac{5306.0}{803.0}$	$\frac{3687.4}{644.3}$
56-57	$\frac{5422.0}{771.0}$	$\frac{4329.5}{628.3}$

In table I-6 are demonstrated the calculated results of the pollutant mixture air+ styrene+ acetone with C_{in}^{Ac} =750 mg.m^{-3}= constant. The results are calculated from the average of overall experimental results in this part. In the section I.1.3 is represented the calculated examples for the parameters in the next table.

Table I-6- Calculated results for the pollutant styrene and acetone (C_{in}^{Ac}=750 mg.m^{-3}).

Time after inoculation (day)	$OL\frac{Sty}{Ac}$ *(g.m^{-3}.h^{-1})*	OL_w *(g.m^{-3}.h^{-1})*	$RE\frac{Sty}{Ac}$ *(%)*	RE_w *(%)*	$EC\frac{Sty}{Ac}$ *(g.m^{-3}.h^{-1})*	EC_w *(g.m^{-3}.h^{-1})*
47-48	$\frac{28.9}{33.7}$	62.6	$\frac{96.6}{28.2}$	86.8	$\frac{27.9}{26.3}$	54.3
48-49	$\frac{39.9}{35.6}$	75.5	$\frac{96.6}{24.5}$	69.8	$\frac{38.6}{14.3}$	52.9
49-50	$\frac{65.2}{31.9}$	97.0	$\frac{96.2}{25.1}$	73.0	$\frac{62.7}{8.2}$	70.9
50-53	$\frac{114.6}{35.6}$	147.0	$\frac{61.4}{23.9}$	53.2	$\frac{70.1}{8.3}$	78.4
53-54	$\frac{132.5}{35.1}$	167.6	$\frac{58.7}{21.0}$	50.8	$\frac{77.8}{7.5}$	85.3
54-55	$\frac{193.8}{33.5}$	227.4	$\frac{42.1}{19.1}$	38.7	$\frac{81.5}{6.4}$	87.9
56-56	$\frac{225.3}{34.1}$	259.4	$\frac{30.5}{19.5}$	29.1	$\frac{68.7}{6.8}$	75.5
56-57	$\frac{229.4}{32.9}$	259.5	$\frac{26.1}{27.1}$	26.1	$\frac{60.8}{8.7}$	68.8

II. Annexes II

II.1 Examples of dry mass calculation

In order to calculate the dry mass, the biomass in the reactor was centrifuged, putted in a drying- oven and weighted.

Afterwards is represented one example of dry mass calculation (example: 29.58 day).

- $m_{dry\ mass} = 0.01466\ g$ in dishes.
- Each dish was 1.5 ml.
- Biomass placed in volumetric flask with 10.00 ml.
- 20.00 ml sample of biomass.

$$m_{dry\ mass} = 0.01466g$$
$$V_{volumetric\ dishes} = 10.00ml$$
$$C_{dry\ mass} = \frac{m_{dry\ mass} \times V_{d\ ishes}}{V_{volumetric\ flask}} = \frac{0.01466 \times 1.5}{10.00} = 0.0977g/10.00ml$$
$$V_{sample} = 20.00ml$$
$$C_{dry\ mass} = 0.0977g/20.00ml$$
$$for \quad 1000ml$$
$$C_{dry\ mass} = \frac{C_{dry\ mass} \times V_{s\ ample}}{V_{1000ml}} = \frac{0.0977 \times 20.00}{1000.0} = 4.887g/L$$

In table II-1 (figure 4-10), are demonstrated the experimental results of the X_f and X_{dm}. In this table are represented only the X_f and X_{dm}, average results, not the overall results.

Table II-1- Biomass changes during the reactor operation

Time of operation (day)	X_{dm} $(g.L^{-1})$	X_f $(g.L^{-1})$
0.00	0,305	0.306
19.57	4,148	2.28
20.59	4,074	2.38
21.57	3,595	3.00
25.58	4,844	3.09
27.59	3,844	3.97
29.58	4,887	6.14
34.57	3,644	5.75
35.60	4,044	5.72
36.58	2,522	2.79
40.61	3,528	5.30
42.62	3,528	7.01
47.57	4,411	5.95
49.63	5,133	7.09
53.58	5,294	7.09
55.57	5,383	7.68
57.59	5,478	6.44

III. Annexes II

III.1 Safety data sheet

III.1.1 Acetone

Material Safety Data Sheet

Revision Issued: 12/12/2008 Supercedes: 10/13/2005 First Issued: 3/04/87

Section I - Chemical Product And Company Identification

Product Name: Acetone

CAS Number: 67-64-1 HBCC MSDS No. CA02000

HILL BROTHERS Chemical Co.

1675 No. Main Street, Orange, California 92867
Telephone No: 714-998-8800 | Chemtrec: 800-424-9300

Section II - Composition/Information On Ingredients

			Exposure Limits (TWAs) in Air		
Chemical Name	CAS Number	%	ACGIH TLV	OSHA PEL	STEL
Acetone	67-64-1	100	500 ppm	1000 ppm	750 ppm

Section III - Hazard Identification

Routes of Exposure: Inhalation, skin absorption, ingestion, or eye contact.
Summary of Acute Health Hazards
Ingestion: Acetone has a low order of toxicity but is very irritating to mucous membranes. Ingestion of a toxic dose can cause gastroenteric irritation, narcosis and injury to the kidneys and liver. Aspiration into lungs can produce severe lung damage and is a medical emergency. Other symptoms are expected to parallel inhalation. Large amounts can cause dehydration.
Inhalation: The vapor is irritating to mucous membranes. Vapor concentration of 2,500-3,000 ppm causes minor irritation of eyes, nose and throat. Inhalation of higher concentration may cause headache, nausea, confusion, drowsiness, convulsions and coma. Higher concentrations can produce central nervous system depression, narcosis, and unconsciousness.
Skin: Prolonged exposure to the vapor irritates the skin. Repeated and prolonged contact of the liquid with skin can cause dryness and erythema (inflammation).
Eyes: Eye contact with acetone is irritating and may be damaging. Produces irritation, characterized by a burning sensation, redness, tearing, inflammation, and possible corneal injury.
Summary of Chronic Health Hazards: N/A
Effects of Overexposure: Acts as an anesthetic in very high concentrations. Headache, nausea, dizziness, and narcosis can result from excessive exposure to vapors. Causes severe eye irritation, experienced as discomfort or pain, excess blinking and tear production, with marked excess redness and swelling of the conjunctiva. Corneal injury may occur. Prolonged contact of the liquid with the skin can have a defatting action and may result in dermatitis. Absorption through intact skin is not expected to cause systemic injury; however, possible skin absorption should be considered in meeting TLV requirements.

Product Name: Acetone 1

(Continuation)

Medical Conditions Generally Aggravated by Exposure: Use of alcoholic beverages enhances toxic effects. Exposure may increase the toxic potential of chlorinated hydrocarbons, such as chloroform, trichloroethane.

Note to Physicians: Aspirated acetone may cause severe lung damage and present a significant hazard. Stomach contents should be evacuated quickly in a manner which avoids aspiration. Otherwise, treatment of overexposure is directed at the control of symptoms and the clinical condition of the patient. No specific antidote is known.

Section IV - First Aid Measures

Ingestion: Do not make an unconscious person vomit. If conscious give 2 glasses of water to dilute. DO NOT INDUCE VOMITING. GET MEDICAL ATTENTION IMMEDIATELY. If vomiting occurs, keep head below hips to prevent aspiration into the lungs.

Inhalation: Remove to fresh air. Administer artificial respiration if breathing is irregular or stopped. If breathing is difficult, oxygen may be given by qualified personnel. GET MEDICAL ATTENTION.

Skin: Wash with large quantities of water and soap or a mild detergent. Remove contaminated clothing. Seek medical attention if irritation from contact persists.

Eyes: Flush eyes with water immediately for at least 15 minutes, lifting the upper and lower lids. GET MEDICAL ATTENTION, preferably from an ophthalmologist.

Section V - Fire Fighting Measures

Flash Point: -4°F (-20°C) **Autoignition Temperature:** 869°F (465°C)

Lower Explosive Limit: 2.5 **Upper Explosive Limit:** 12.8

Unusual Fire and Explosion Hazards: Acetone is extremely flammable. Vapors form from this product and may travel or be moved by air currents and ignited by pilot lights, other flames, smoking, sparks, heaters, electrical equipment, static discharges or other ignition sources at locations distant from product handling point. Vapors from this material may settle in low or confined areas or travel a long distance to an ignition source and flash back explosively. This material may produce a floating fire hazard.

Extinguishing Media: Small fire: Use carbon dioxide or dry chemical. Large fire: Use polar solvent (alcohol) type foam. The normal firefighting foams that are suitable for gasoline or hydrocarbon fires will break down and will not extinguish acetone fires. Water spray will reduce the intensity of flames. Acetone/water solutions have flash points when the acetone concentration is greater than 8% (by weight). The fire point, which is the percent by weight when a solution sustains a flame, is higher than that.

Special Firefighting Procedures: The use of self-contained breathing apparatus is recommended for fire fighters. Use water spray to cool fire-exposed containers and to dilute and reduce fire intensity. Use remote spray monitors or fight fire from behind shields. Use water spray to disperse vapors; re-ignition is possible.

Section VI - Accidental Release Measures

Provide maximum explosion-proof ventilation. Eliminate all sources of ignition. Flush spilled material into suitable retaining areas or containers with large quantities of water. Small amounts of spilled material may be absorbed into an appropriate absorbent.

Product Name: Acetone 2

(Continuation)

Section VII - Handling and Storage

Handling and Storing Precautions: Store in a cool, clean, well-ventilated fireproof storage room or cabined to meet OSHA requirements. Sprinkler fire protection is needed in areas of storage, handling and use. Acetone must be stored and handled away from heat. Do not pressurize, cut, weld, braze, solder, drill, grind, or expose empty containers to heat, sparks or open flames. Electrically interconnect and ground containers for all transfers of acetone to avoid fires from static sparks. Avoid breathing vapor.
Other Precautions: Transfer hazard: Vapors of this product may be ignited by static sparks. Use proper bonding and grounding during liquid transfer as described in National Fire Protection Association document NFPA 77.

Section VIII - Exposure Controls/Personal Protection

Respiratory Protection: Use only NIOSH- or MSHA -approved respirators. For a non-routine or emergency exposure above the TLV, use a full facepiece gas mask with organic vapor canister, or an air-supplied respirator in accordance with conditions. Use self-contained breathing apparatus in high vapor concentrations.
Respirator Selection
5,000 ppm: GMOVc* 20,000 ppm: GMOVfb/SAF/SCBAF* Escape: GMOV/SCBA* *see below
Ventilation: General mechanical ventilation may be sufficient to keep product vapor concentrations within specified time-weighted TLV ranges. Supplemental local exhaust may be required to maintain safe vapor concentrations.
Protective Clothing: Wear appropriate clothing to prevent repeated or prolonged skin contact. The use of impermeable gloves, aprons, boots, and lab coat are advised to prevent skin irritation.
Eye Protection: Safety glasses, chemical goggles, and/or face shields are recommended to safeguard against potential eye contact, irritation, or injury.
Other Protective Clothing or Equipment: Eye washes and safety showers should be readily available in the work areas.
Work/Hygienic Practices: Employees should Wash hands thoroughly with soap and water before eating, drinking, smoking or using toilet facilities. Do NOT place food, coffee or other drinks in the area where dusting or splashing of solutions is possible.

Section IX - Physical and Chemical Properties

Physical State: Liquid
pH: 7
Melting Point/Range: -138°F (-94.4°C)
Boiling Point/Range: 133°F (56.1°C)
Appearance/Color/Odor: Clear, colorless liquid with a sweet, mint-like odor
Solubility in Water: Miscible in all proportions in water
Vapor Pressure (mmHg): 400 @ 104°F; (39.5°C). 181 @ 68°F (20°C)
Specific Gravity (Water=1): 0.791 @ 68°F (20°C)
Molecular Weight: 58.08
Vapor Density (Air=1): 2.0
% Volatiles: 100%
How to detect this compound : N/A
Freezing Point: -94.4°C (-138°F)
Evaporation Rate (Butyl Acetate=1): 5.6

(Continuation)

Section X - Stability and Reactivity

Stability: Stable **Hazardous Polymerization:** Will not occur

Conditions to Avoid: Heat - acetone is a highly flammable material.

Materials to Avoid: Acetone is incompatible with strong oxidizing agents and strong acids or bases. Concentrated nitric and sulfuric acid mixtures, oxidizing materials, chloroform, alkalis, chlorine compounds, acids, potassium t-butoxide.

Hazardous Decomposition Products: Thermal decomposition in the presence of air may yield carbon monoxide and/or carbon dioxide.

Section XI - Toxicological Information

Eye (human) = 500 ppm
Dermal, guinea pig: LD50 = >9400 uL/kg;
Draize test, rabbit, eye: 20 mg Severe;
Draize test, rabbit, eye: 20 mg/24H Moderate;
Draize test, rabbit, eye: 10 uL Mild;
Draize test, rabbit, skin: 500 mg/24H Mild;
Inhalation, mouse: LC50 = 44 gm/m3/4H;
Inhalation, rat: LC50 = 50100 mg/m3/8H;
Oral, mouse: LD50 = 3 gm/kg;
Oral, rabbit: LD50 = 5340 mg/kg;
Oral, rat: LD50 = 5800 mg/kg

Section XII - Ecological Information

Octanol/Water Partition Coefficient: 0.58
Fish: Rainbow trout: 5540 mg/l; 96-hr; LC50Fish: Bluegill/Sunfish: 8300 mg/l; 96-hr

Section XIII - Disposal Considerations

Dispose of acetone in accordance with applicable local, county, state and federal regulations

Section XIV - Transport Information

DOT Proper Shipping Name: Acetone
DOT Hazard Class/ I.D. No.: 3, UN1090, II

Section XV - Regulatory Information

CERCLA (Comprehensive Environmental Response, Compensation, and Liability Act) Hazardous Substance: Acetone, CAS # 67-64-1
5000 Lbs. (2270 Kilograms) (753.01 Gals.) Reportable Quantity (RQ)
RCRA (Resource Conservation & Recovery Act) Hazardous Waste Code:
Acetone, CAS # 67-64-1, U002
Medical Surveillance Suggested: Preplacement examinations should evaluate skin and respiratory conditions. Acetone can be detected in the blood, urine, and expired air and has been used as an index of exposure.
Uniform Fire Code Rating: Class IB Flammable Liquid
NFPA (National Fire Protection Association) Rating:
Health - 1; Flammability - 3; Instability - 0
0=Insignificant 1=Slight 2=Moderate 3=High 4=Extreme
Carcinogenicity Lists:
National Toxicology Program (NTP): No
International Agency for Research on Cancer (IARC) Monograph: No

Product Name: Acetone 4

(Continuation)

Occupational Safety & Health Administration (OSHA) Regulated: No

Section XVI - Other Information

Synonyms/Common Names:
Dimethyl Ketone, Propanone, 2-propanone, Dimethyl Ketal
Chemical Family/Type: Oxygenated Hydrocarbon, Ketone
Section(s) changed since last revision: XV

IMPORTANT! Read this MSDS before use or disposal of this product. Pass along the information to employees and any other persons who could be exposed to the product to be sure that they are aware of the information before use or other exposure. This MSDS has been prepared according to the OSHA Hazard Communication Standard [29 CFR 1910.1200]. The MSDS information is based on sources believed to be reliable. However, since data, safety standards, and government regulations are subject to change and the conditions of handling and use, or misuse are beyond our control, **Hill Brothers Chemical Company** makes no warranty, either expressed or implied, with respect to the completeness or continuing accuracy of the information contained herein and disclaims all liability for reliance thereon. Also, additional information may be necessary or helpful for specific conditions and circumstances of use. It is the user's responsibility to determine the suitability of this product and to evaluate risks prior to use, and then to exercise appropriate precautions for protection of employees and others.

III.1.2 Styrene

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Chemicals & Laboratory Equipment

Health	2
Fire	3
Reactivity	0
Personal Protection	H

Material Safety Data Sheet
Styrene (monomer) MSDS

Section 1: Chemical Product and Company Identification

Product Name: Styrene (monomer)

Catalog Codes: SLS2512, SLU1027

CAS#: 100-42-5

RTECS: WL3675000

TSCA: TSCA 8(b) inventory: Styrene (monomer)

CI#: Not available.

Synonym: Vinylbenzene

Chemical Formula: C8H8

Contact Information:

Sciencelab.com, Inc.
14025 Smith Rd.
Houston, Texas 77396

US Sales: **1-800-901-7247**
International Sales: **1-281-441-4400**

Order Online: ScienceLab.com

CHEMTREC (24HR Emergency Telephone), call:
1-800-424-9300

International CHEMTREC, call: 1-703-527-3887

For non-emergency assistance, call: 1-281-441-4400

Section 2: Composition and Information on Ingredients

Composition:

Name	CAS #	% by Weight
Styrene (monomer)	100-42-5	100

Toxicological Data on Ingredients: Styrene (monomer): ORAL (LD50): Acute: 2650 mg/kg [Rat]. 316 mg/kg [Mouse]. VAPOR (LC50): Acute: 12000 ppm 4 hour(s) [Rat]. 9500 ppm 4 hour(s) [Mouse].

Section 3: Hazards Identification

Potential Acute Health Effects:
Very hazardous in case of eye contact (irritant). Hazardous in case of skin contact (irritant, permeator), of ingestion, of inhalation. Inflammation of the eye is characterized by redness, watering, and itching.

Potential Chronic Health Effects:
CARCINOGENIC EFFECTS: Classified + (PROVEN) by OSHA. Classified 2B (Possible for human.) by IARC. A4 (Not classifiable for human or animal.) by ACGIH.
MUTAGENIC EFFECTS: Not available.
TERATOGENIC EFFECTS: Not available.
DEVELOPMENTAL TOXICITY: Not available.
The substance is toxic to the nervous system, upper respiratory tract.
Repeated or prolonged exposure to the substance can produce target organs damage.

Section 4: First Aid Measures

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(Continuation)

Eye Contact:
Check for and remove any contact lenses. Immediately flush eyes with running water for at least 15 minutes, keeping eyelids open. Cold water may be used. Do not use an eye ointment. Seek medical attention.

Skin Contact:
After contact with skin, wash immediately with plenty of water. Gently and thoroughly wash the contaminated skin with running water and non-abrasive soap. Be particularly careful to clean folds, crevices, creases and groin. Cover the irritated skin with an emollient. If irritation persists, seek medical attention. Wash contaminated clothing before reusing.

Serious Skin Contact:
Wash with a disinfectant soap and cover the contaminated skin with an anti-bacterial cream. Seek immediate medical attention.

Inhalation: Allow the victim to rest in a well ventilated area. Seek immediate medical attention.

Serious Inhalation: Not available.

Ingestion:
Do not induce vomiting. Examine the lips and mouth to ascertain whether the tissues are damaged, a possible indication that the toxic material was ingested; the absence of such signs, however, is not conclusive. Loosen tight clothing such as a collar, tie, belt or waistband. If the victim is not breathing, perform mouth-to-mouth resuscitation. Seek immediate medical attention.

Serious Ingestion: Not available.

Section 5: Fire and Explosion Data

Flammability of the Product: Flammable.

Auto-Ignition Temperature: 490°C (914°F)

Flash Points: CLOSED CUP: 31.1°C (88°F). (Cleveland) OPEN CUP: 36.7°C (98.1°F) (TAG).

Flammable Limits: LOWER: 1.1% UPPER: 6.1%

Products of Combustion: These products are carbon oxides (CO, CO2).

Fire Hazards in Presence of Various Substances:
Flammable in presence of open flames and sparks.
Slightly flammable to flammable in presence of heat.

Explosion Hazards in Presence of Various Substances:
Risks of explosion of the product in presence of mechanical impact: Not available.
Risks of explosion of the product in presence of static discharge: Not available.

Fire Fighting Media and Instructions:
Flammable liquid, soluble or dispersed in water.
SMALL FIRE: Use DRY chemical powder.
LARGE FIRE: Use alcohol foam, water spray or fog. Cool containing vessels with water jet in order to prevent pressure build-up, autoignition or explosion.

Special Remarks on Fire Hazards: Not available.

Special Remarks on Explosion Hazards: Not available.

Section 6: Accidental Release Measures

Small Spill: Absorb with an inert material and put the spilled material in an appropriate waste disposal.

(Continuation)

Large Spill:
Flammable liquid.
Keep away from heat. Keep away from sources of ignition. Stop leak if without risk. Absorb with DRY earth, sand or other non-combustible material. Do not touch spilled material. Prevent entry into sewers, basements or confined areas; dike if needed. Eliminate all ignition sources. Be careful that the product is not present at a concentration level above TLV. Check TLV on the MSDS and with local authorities.

Section 7: Handling and Storage

Precautions:
Keep locked up Keep away from heat. Keep away from sources of ignition. Ground all equipment containing material. Do not ingest. Do not breathe gas/fumes/ vapour/spray. In case of insufficient ventilation, wear suitable respiratory equipment If ingested, seek medical advice immediately and show the container or the label. Avoid contact with skin and eyes

Storage:
Flammable materials should be stored in a separate safety storage cabinet or room. Keep away from heat. Keep away from sources of ignition. Keep container tightly closed. Keep in a cool, well-ventilated place. Ground all equipment containing material. A refrigerated room would be preferable for materials with a flash point lower than 37.8°C (100°F).

Section 8: Exposure Controls/Personal Protection

Engineering Controls:
Provide exhaust ventilation or other engineering controls to keep the airborne concentrations of vapors below their respective threshold limit value. Ensure that eyewash stations and safety showers are proximal to the work-station location.

Personal Protection:
Splash goggles. Lab coat. Vapor respirator. Be sure to use an approved/certified respirator or equivalent. Gloves.

Personal Protection in Case of a Large Spill:
Splash goggles. Full suit. Vapor respirator. Boots. Gloves. A self contained breathing apparatus should be used to avoid inhalation of the product. Suggested protective clothing might not be sufficient; consult a specialist BEFORE handling this product.

Exposure Limits:
TWA: 50 STEL: 100 (ppm)
TWA: 213 STEL: 426 (mg/m3)
Consult local authorities for acceptable exposure limits.

Section 9: Physical and Chemical Properties

Physical state and appearance: Liquid. (Clear viscous liquid.)

Odor: Sweetish. Aromatic.

Taste: Not available.

Molecular Weight: 104.14 g/mole

Color: Colorless.

pH (1% soln/water): Not available.

Boiling Point: 145.2°C (293.4°F)

(Continuation)

Melting Point: -30.6°C (-23.1°F)

Critical Temperature: Not available.

Specific Gravity: 0.906 (Water = 1)

Vapor Pressure: 4.5 mm of Hg (@ 20°C)

Vapor Density: 3.59 (Air = 1)

Volatility: Not available.

Odor Threshold: 0.1 ppm

Water/Oil Dist. Coeff.: The product is equally soluble in oil and water; log(oil/water) = 0

Ionicity (in Water): Not available.

Dispersion Properties: Not available.

Solubility: Very slightly soluble in cold water.

Section 10: Stability and Reactivity Data

Stability: The product is stable.

Instability Temperature: Not available.

Conditions of Instability: Not available.

Incompatibility with various substances: Not available.

Corrosivity: Non-corrosive in presence of glass.

Special Remarks on Reactivity: Not available.

Special Remarks on Corrosivity: Not available.

Polymerization: No.

Section 11: Toxicological Information

Routes of Entry: Dermal contact. Eye contact. Inhalation. Ingestion.

Toxicity to Animals:
WARNING: THE LC50 VALUES HEREUNDER ARE ESTIMATED ON THE BASIS OF A 4-HOUR EXPOSURE.
Acute oral toxicity (LD50): 316 mg/kg [Mouse].
Acute toxicity of the vapor (LC50): 9500 ppm 4 hour(s) [Mouse].

Chronic Effects on Humans:
CARCINOGENIC EFFECTS: Classified + (PROVEN) by OSHA. Classified 2B (Possible for human.) by IARC.
A4 (Not classifiable for human or animal.) by ACGIH.
The substance is toxic to the nervous system, upper respiratory tract.

Other Toxic Effects on Humans: Hazardous in case of skin contact (irritant, permeator), of ingestion, of inhalation.

Special Remarks on Toxicity to Animals: Not available.

Special Remarks on Chronic Effects on Humans:

(Continuation)

Animal embryotoxic. Postnatal development injury in animal. Menstrual disorders in human. Human: passes the placental barrier, detected in maternal milk.

Special Remarks on other Toxic Effects on Humans: Not available.

Section 12: Ecological Information

Ecotoxicity: Not available.

BOD5 and COD: Not available.

Products of Biodegradation:
Possibly hazardous short term degradation products are not likely. However, long term degradation products may arise.

Toxicity of the Products of Biodegradation: The products of degradation are more toxic.

Special Remarks on the Products of Biodegradation: Not available.

Section 13: Disposal Considerations

Waste Disposal:

Section 14: Transport Information

DOT Classification: Class 3: Flammable liquid.

Identification: : Styrene monomer, inhibited : UN2055 PG: III

Special Provisions for Transport: Marine Pollutant

Section 15: Other Regulatory Information

Federal and State Regulations:
Pennsylvania RTK: Styrene (monomer)
Florida: Styrene (monomer)
Minnesota: Styrene (monomer)
Massachusetts RTK: Styrene (monomer)
New Jersey: Styrene (monomer)
TSCA 8(b) inventory: Styrene (monomer)
SARA 313 toxic chemical notification and release reporting: Styrene (monomer)
CERCLA: Hazardous substances.: Styrene (monomer)

Other Regulations: OSHA: Hazardous by definition of Hazard Communication Standard (29 CFR 1910.1200).

Other Classifications:

WHMIS (Canada):
CLASS B-2: Flammable liquid with a flash point lower than 37.8°C (100°F).
CLASS D-2A: Material causing other toxic effects (VERY TOXIC).

DSCL (EEC):
R10- Flammable.
R38- Irritating to skin.
R41- Risk of serious damage to eyes.
R45- May cause cancer.

HMIS (U.S.A.):

(Continuation)

Health Hazard: 2

Fire Hazard: 3

Reactivity: 0

Personal Protection: h

National Fire Protection Association (U.S.A.):

Health: 2

Flammability: 3

Reactivity: 2

Specific hazard:

Protective Equipment:
Gloves.
Lab coat.
Vapor respirator. Be sure to use an approved/certified respirator or equivalent. Wear appropriate respirator when ventilation is inadequate.
Splash goggles.

Section 16: Other Information

References: Not available.

Other Special Considerations: Not available.

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Last Updated: 11/06/2008 12:00 PM

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III.1.3 Potassium hydroxide

QUIP LABORATORIES INCORPORATED

QUIP SERVICE SELLS QUIP

MATERIAL SAFETY DATA SHEET

SECTION 1: CHEMICAL PRODUCT AND COMPANY IDENTIFICATION:

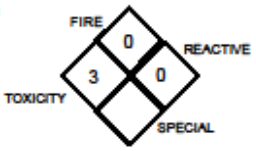
Manufactured by:
Quip Laboratories, Inc.
1500 Eastlawn Avenue
Wilmington, DE 19802
Non-Emergency: (302) 761-2600

CHEMICAL EMERGENCY NUMBER
CHEMTREC
1-800-424-9300

Product Name:	**ENVIRO-KLEEN 100**
Common Name:	Potassium Hydroxide Mixture
Chemical Name:	Chemical Mixture
Formula:	Chemical Mixture
Product Use:	High Alkaline Non-Foaming Cleaner

HAZARD RATING

4-EXTREME
3-HIGH
2-MODERATE
1-SLIGHT
0-INSIGNIFICANT



SECTION 2: COMPOSITION / INFORMATION ON INGREDIENTS:

INGREDIENTS:		% / Wt.	TLV/ACGIH
Water	(C.A.S.# 7732-18-5)	To qs%	None Established
Potassium Hydroxide	(C.A.S.# 1310-58-3)	10.0 - 15.0	2 mg/m^3
Ethylenediamine tetra-acetic Acid, Sodium salt	(C.A.S. # 64-02-8)	10.0 - 20.0	None Established
Amphoteric Surfactant	(C.A.S.# Mixture)	Prop.	None Established
Proprietary Ingredients	(C.A.S. # N/A)	Prop.	N/A

Note: (Proprietary ingredient information available upon written request and agreement of confidentiality.)

SECTION 3: HEALTH HAZARD IDENTIFICATION DATA:

MOST IMPORTANT HAZARDS: Corrosive. Can cause burns, even permanent damage to eyes, respiratory system and skin. Harmful if swallowed. May cause pain, nausea, vomiting and diarrhea.

ROUTES OF ENTRY: Eyes; Yes Skin; Yes Inhalation; Yes Ingestion; Yes

POTENTIAL HEALTH EFFECTS:

EYE CONTACT: Causes severe burns or even permanent damage. Severity depends upon concentration and duration of exposure.

SKIN CONTACT: Causes skin burns. May cause more severe response if confined to skin, or if skin is abraded, scratched or cut.

INHALATION: Exposure to mists generated from this material causes irritation, or even burns to the respiratory tract. May result in coughing, difficulty breathing and sore throat.

INGESTION: Harmful if swallowed. May cause pain, nausea, vomiting, diarrhea, weakness and fatigue.

CARCINOGENICITY: NTP; No IARC; No OSHA; No ACGIH: No

CONDITIONS AGGRAVATED BY EXPOSURE: None known.

SECTION 4: EMERGENCY & FIRST AID PROCEDURES:

EYE CONTACT: Immediately flush eyes with a directed stream of water for at least 15 minutes, forcibly holding eyelids apart to ensure complete irrigation of all eye and lid tissues. Washing eyes within several seconds of exposure is essential to achieve maximum effectiveness. Get immediate medical attention.

(Continuation)

PRODUCT CODE: EK100 PRODUCT NAME: Enviro-Kleen 100

SECTION 4: EMERGENCY & FIRST AID PROCEDURES (Continued):

SKIN CONTACT: Immediately flush contaminated area with water. Remove contaminated clothing and footwear. Wash contaminated areas with plenty of soap and water. Wash contaminated clothing before re-use. Discard footwear that cannot be decontaminated. Get immediate medical attention
INHALATION: Remove to fresh air if safe to transport. Otherwise attempt to provide fresh are by ventilation. If breathing is difficult, have a trained person administer oxygen. If not breathing, first call 911, then give artificial respiration.
INGESTION: DO NOT INDUCE VOMITING. Give large quantities of water (If available, give several glasses of milk to dilute). If vomiting occurs spontaneously, keep airway clear and give more water. Never give anything by mouth to an unconscious person. Get immediate medical attention.
NOTES TO PHYSICIAN: No specialized procedures. Treat for clinical symptoms.

SECTION 5: FIRE FIGHTING MEASURES:

FLASH POINT: Not Applicable **AUTO IGNITION:** Not Applicable **LEL / UEL**: Not Applicable

EXTINGUISHING MEDIA: Use agents appropriate for surrounding fire. May react with water with the generation of heat.
FIRE FIGHTING PROCEDURES: Wear NIOSH/MSHA positive-pressure, self contained breathing apparatus and full protective clothing.
FIRE AND EXPLOSION HAZARD: None known.

SECTION 6: ACCIDENTAL RELEASE MEASURES:

PERSONAL PRECAUTIONS: Follow protective measures provided under Personal Protection in Section 8.
Evacuate unnecessary personnel and eliminate all sources of ignition. Contain Spill.
ENVIRONMENTAL PRECAUTIONS: Do not allow entry into sewers and waterways.
METHODS FOR CLEANING UP: For small spills, soak up with absorbent material and place in properly labeled containers for disposal.
For large spills, dike and pump into properly labeled containers for reclamation or disposal, according to local, state, and federal regulations.
WASTE DISPOSAL METHODS: Dispose of in a landfill or flush to a sanitary sewer in accordance with local, state and federal regulations.

SECTION 7: HANDLING AND STORAGE:

HANDLING: Use with adequate ventilation. Avoid breathing vapors. Wear personal protection equipment as described in Exposure Controls/Personal Protection (Section 8) of the MSDS. Never move drums with open bungs.

SPECIAL MIXING AND HANDLING INSTUCTIONS: Do not allow contact with materials as noted in Section 10.

STORAGE: Keep container tightly closed and properly labeled. Do not store in aluminum containers or use aluminum fittings or transfer lines, as flammable hydrogen gas can be generated.

SECTION 8: EXPOSURE CONTROL / PERSONAL PROTECTION INFORMATION:

ENGINEERING CONTROLS: Handle product in a well ventilated area. If product is handled in an open system, the use of process closures, local exhaust, ventilation, and/or other engineering controls should be considered to control airborne levels to below recommended exposure limits, or below acceptable levels where there are no limits.
PERSONAL PROTECTION:
RESPIRATORY: A NIOSH approved respirator with a dust, fume and mist filter may be permissible under certain circumstances where airborne concentrations are expected to exceed exposure limits, or when symptoms have been observed that are indicative of overexposure.
A respiratory protection program that meets 29CFR 1910.134 and ANSI Z88.2 requirements must be followed whenever workplace conditions warrant use of a respirator.

(Continuation)

PRODUCT CODE: EK100 PRODUCT NAME: Enviro-Kleen 100

SECTION 8: EXPOSURE CONTROL / PERSONAL PROTECTION INFORMATION (Continued):

EYE/FACE: Wear chemical safety goggles plus full face shield to protect against contact when appropriate. (ANSIZ87.1)
SKIN: Wear protective clothing to minimize skin contact. Wear chemical resistant glove such as rubber, neoprene or vinyl.
OTHER: Discard leather items that cannot be decontaminated. Emergency shower and Eyewash facility should be in close proximity (ANSI Z358.1).

SECTION 9: PHYSICAL AND CHEMICAL PROPERTIES:

APPEARANCE: Clear Blue Liquid
SPECIFIC GRAVITY: 1.17
SOLUBILITY IN WATER: Completely
VAPOR PRESSURE: N/A
VAPOR DENSITY (AIR = 1): Not Applicable
ODOR: Mild Musty Odor.
pH: (1% solution in water) 12.5
EVAPORATION RATE: Not Applicable
BOILING POINT: Not Established

SECTION 10: STABILITY & REACTIVITY DATA:

INCOMPATIBLE MATERIALS: Product is corrosive to soft metals such as tin, aluminum, zinc, copper and brass, or alloys containing these metals. Avoid contact with leather, wool, acids, organic halogen compounds, or organic nitro compounds.
STABILITY: Product is stable.
HAZARDOUS POLYMERIZATION: Will not occur.
HAZARDOUS DECOMPOSITION PRODUCTS: None.

SECTION 11: TOXICOLOGICAL INFORMATION:

POTASSIUM HYDROXIDE (KOH) CAS# 1310-58-3: This substance is alkaline and corrosive. Minimize contact. The irritating and corrosive properties of this product depend on its concentration. It is toxic by the oral route. It may cause burns and other effects to the mucus membranes, mouth and digestive tracts. Its dermal toxicity has not been determined. It may cause burns that are not immediately noticed or painful. Inhalation of dust or vapors can cause airway effects including burns. This product is irritating and corrosive to eyes and skin.
The irritating and corrosive properties of this material depend on its concentration. In general, serious injury is associated with products with a pH of 11.5 or higher.

SECTION 12: ECOLOGICAL INFORMATION:

POTASSIUM HYDROXIDE (KOH) CAS# 1310-58-3:
TOXICITY: This material is believed to be slightly toxic to aquatic life.
PERSISTANCE: This material is believed to be unlikely to persist in the environment.
BIOACCUMULATION: This material is believed to be unlikely to bioaccumulate.
BIODEGRADABILITY: All components are biodegradable.

SECTION 13: DISPOSAL CONSIDERATIONS:

POTASSIUM HYDROXIDE (KOH) CAS# 1310-58-3:
Dispose of all waste and contaminated equipment in accordance with all applicable Federal, State and Local health and environmental regulations.

(Continuation)

PRODUCT CODE: EK100 PRODUCT NAME: Enviro-Kleen 100

SECTION 14: TRANSPORT INFORMATION:

INTERNATIONAL: UN Number: UN1760 (Ground only) / UN1760 (Air)

US TRANSPORTATION REGULATIONS:

DOT Classification: NA1760, 8 (Corrosive)
DOT Proper Shipping Name: Compounds, cleaning liquid (Contains Potassium hydroxide)
Packing Group: III

CANADIAN TRANSPORTATION OF DANGEROUS GOODS:
N/A

SECTION 15: REGULATORY INFORMATION:

US FEDERAL REGULATIONS:
TSCA: In TSCA (Toxic Substances Control Act)

SARA 311 and 312 HAZARD CATEGORIES:

Immediate (Acute): Yes Delayed (Chronic): No Fire: No
Reactivity: Yes Sudden Release of Pressure: No

SARA SECTION 313 NOTIFICATION:
This product does contain toxic chemicals subject to the reporting requirements of Section 313 of Title III of the Superfund Amendments and Reauthorization Act of 1986 and 40 CFR Part 372.

CAA 602 OZONE DEPLETING SUBSTANCES (ODS):
This product neither contains nor is manufactured with an ozone depleting substance subject to the labeling requirements of the Clean Air Act Amendments 1990 and 40 CFR Part 82.

VOLATILE ORGANIC COMPOUNDS (VOC): Not Applicable.

US STATE REGULATIONS:
VOLATILE ORGANIC COMPOUNDS (CARB): Not Applicable.

CANADIAN REGULATIONS: N/A
DSL/NDSL: N/A
WHMIS CLASSIFICATION: N/A

SECTION 16: SPECIAL INFORMATION:

The information in this Material Safety Data Sheet should be provided to all who use, handle, transport, or otherwise are exposed to this product. This information has been prepared for the guidance of plant engineering, operations, management and persons working with or handling this product. The information presented in this MSDS is premised upon proper handling and anticipated uses, and is for the material without chemical additions/alterations. Additionally, if this Material Safety Data Sheet is more than three years old, please contact this supplier at the phone number above Section 1 to make sure this sheet is current.

PREPARED BY: D. M. McFadden DATE: 5/9/2006