İSTANBUL TECHNICAL UNIVERSITY ★ INSTITUTE OF SCIENCE AND TECHNOLOGY

THE EFFECT OF OZONE PRETREATMENT ON THE BIODEGRADABILITY AND TOXICITY OF NATURAL TANNIN AND SYNTHETIC TANNIN

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Programme: Environmental Biotechnology

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PREFERENCES

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NOTATION LIST

BOD_5	: Biochemical oxygen demand [ML^{-1}]
COD	: Chemical oxygen demand [ML^{-1}]
F/M ratio	: Food to microorganisms ratio
S_O	: Oxygen concentration [ML^{-1}]
S_S	: Readily biodegradable substrate concentration [MCODL^{-1}]
X_H	: Concentration of heterotrophic biomass [ML^{-1}]
Y_H	: Growth yield [MCODMCOD^{-1}]
Y_{STO}	: Storage yield [MCODMCOD^{-1}]
S_{PG}	: residual soluble microbial end products generated in glucose reactor [MCODL^{-1}]
S_{G0}	: initial soluble COD of glucose reactor [MCODL^{-1}]
S_{T0}	: initial soluble COD of wastewater reactor [MCODL^{-1}]
S_{S0}	: initial soluble biodegradable COD of wastewater reactor [MCODL^{-1}]
S_T	: final soluble COD of wastewater reactor [MCODL^{-1}]
S_I	: initial soluble inert COD of wastewater reactor [MCODL^{-1}]
Y_{SP}	: ratio between the soluble residual products and biodegradable COD

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THE EFFECT OF OZONE PRETREATMENT ON THE BIODEGRADABILITY AND TOXICITY OF NATURAL TANNIN AND SYNTHETIC TANNIN

SUMMARY

The textile industry is a water intensive industry and finishing processes are the major water consuming part of this industry. The textile dyeing and finishing processes are characterized by its huge water requirement and also variety and complexity of process chemicals employed. The recalcitrance of the final effluent usually originates from the considerable amounts of dyestuffs and auxiliaries. Although they are not in the list of priority pollutants, dyestuffs and auxiliaries are known for their poor biodegradability and potential toxicity. In present study two frequently used textile tannins; i.e. natural tannin and synthetic tannin (NT and ST) are used. Natural and synthetic tannins are being frequently applied for the attachment of acid or metal complex dyestuffs onto polyamide fibers. They are sources of recalcitrance and toxicity in dyehouse effluent.

First of all inert COD tests were carried out on raw samples. Results have indicated that raw NT did not have a strength inert COD value; however, ST had an inert COD value of 135mg/l. The toxicity tests performed with *Phaeodactylum tricornutum* pure culture and conducted with both raw tannins. Results were demonstrated that both raw tannins are extremely toxic. According to the toxicity test results carried out for raw tannins ozone pretreatment was applied for NT and ST. The experiments were evaluated at this conditions: Ozone Feed Rate = 1000 mg/h; pH = 3.5; t = 40 min. The effects of this pretreatment on the biodegradability and toxicity of tannins were assessed. After ozonation pretreatment, inert COD of NT was decreased to 9mg/l, and inert COD of ST was decreased to 41mg/l. After the application of ozonation; EC_{80} value for NT was increased to 28 %; and EC_{80} value for ST was increased to 34%. By the aid of sequential filtration/ultra filtration experiments the COD distribution of different molecular weight cut-offs for the raw and ozonated tannins were observed. Then, the obtained results were compared with NT and ST solutions.

SENTETİK VE DOĞAL TANNİNİN OZONLA ÖNARITIMININ BİOLOJİK AYRIŞABİLİRLİK VE TOKSİSİTE ÜZERİNE ETKİLERİ

ÖZET

Türkiye'deki başlıca endüstrilerden biri olan tekstil endüstrisi önemli miktarda enerji, su ve kimyasal tüketiminin olduğu bir endüstri dalıdır ve bu atıksuların büyük bir kısmı boyama işlemlerinden gelmektedir. Kullanılan tekstil hazırlama, boyama ve apreleme işlemlerine ve elde edilmek istenen ürünlere bağlı olarak bir çok yardımcı kimyasal kullanılmaktadır. Kullanılan kimyasalların çoğu, kompleks yapıda, biyolojik arıtmaya dirençli, çoğunlukla zehirli ve refrakter karakterdedir. Özellikle tekstil endüstrisi son işlemlerinde kullanılan bazı yardımcı maddeler hem düşük düzeyde biyolojik ayrışabilirlikleri nedeniyle arıtma tesislerinde sorun çıkartabilmekte, hem de çeşitli organizmalar üzerinde zehirli etki yapmaktadır. Bu olumsuz sonuçları öngörebilmek ve negatif etkileri aza indirebilmek için yardımcı kimyasallar bilimsel olarak araştırılmalıdır.Bu çalışmada, tekstil endüstrisinde yaygın olarak asit ve metal kompleks boyama işlemlerinde uygulanan, boyaların naylon kumaşta tutunabilmesi için kullanılan doğal ve sentetik taninnler (DT ve ST) incelenmiştir.

Öncelikle, inert KOİ deneyleri ham numunelere uygulanmıştır. Bu deney sonuçlarında; DT' nin inert KOİ içeriğinin çok önemli değerlerde olmadığı saptanmıştır; fakat ST' in inert KOİ içeriği 135mg/l'dir. Bir sonraki adımda alıcı ortam kalitesinin korunması için *Phaeodactylum tricornutum* (diatom) saf kültürü ile toksisite çalışmaları yapılmıştır. Ham DT ve ST numunelerinin EC değerleri % 1'in altında çıkmıştır. Bu sonuçlardan yola çıkılarak DT ve ST için ön arıtma olarak ozonlama uygulanmıştır. Ozonlama deneyleri 1000 mg/saat ozon dozunda, 3.5 pH da 40 dakika boyunca yürütülmüştür. Bu ön arıtmanın arıtılabilirlik ve zehirlilik üzerine etkisi incelenmiştir. Ozonlamadan sonra alınan numunelerde yürütülen inert KOİ sonuçlarında DT' nin inert KOİ içeriğinin 9mg/l; ST' nin inert KOİ içeriğinin 41mg/l değerlerine düştüğü gözlenmiştir. Ozonlanmış numuneler için zehirlilik testleri uygulandığında da EC_{80} değeri doğal tannin için % 28'e, sentetik tannin için de % 34'e yükselmektedir. Ham ve ozonlanmış tanninler ultrafiltrasyondan geçirilerek çeşitli moleküler boyut dağılım aralıklarında KOİ değerleri elde edilmiştir. Ultrafiltrasyon deneylerinde, farklı gözenek çaplarına sahip filtreler kullanılarak KOİ yükünün azaltılması sağlanmıştır. En son olarak da ham ve ozonlanmış numuneler için uygulanan deneylerin değerlendirmeleri yapılmıştır.

1. INTRODUCTION

1.1 Importance of the Study

Textile industry is one of the most important sectors in the Turkish economy in terms of GDP (Gross Domestic Product), employment and exports. Turkey has become the EU's number three clothing supplier (behind China/ Hong Kong, and Italy) along with Mexico and the number one textile supplier. The textile industry has a great contribution to the Turkish economy, providing about 10% of GDP (Gross Domestic Product), 18 % of industrial production, 20% of manufacturing labour force, and 32% of total Turkish export according to 2003 Figures (ITKB, 2003).

As one of the most energy and water demanding industries, the textile sector is coming face to face with serious environmental pollution concerns (Correia et al., 1994). Water is used extensively throughout textile-processing operations, and water consumption changes according to the operations. The primary pollution source of this industry is described as aqueous effluent of textile mills (US EPA 1997a).

Textile wastewaters are generated from operations such as yarn and fabric preparation, de-sizing, scouring, bleaching, dyeing, finishing etc. As the great range of dyestuffs (major sources of heavy metals, salt, adsorbable organic halogens (AOX) and color in dyehouse effluent), fibres, process auxiliaries (surfactants, tannins, dye carriers, wetting, decreasing, sizing and sequestering agents) and finishing chemicals (flame retardants, antipilling, antimyte, antifungal, antimicrobial preparations) are involved in textile manufacturing, they generate various volumes of wastewater with complex nature (US EPA, 1997b).

The name "xenobiotic" is given to most of these auxiliaries since they can be classified as synthetic chemicals that resist conventional aerobic biodegradation. Moreover they may create problems in textile wastewater treatment plants, in publicity owned sewage treatment plant and receiving water bodies. Most mills activate their own wastewater treatment or pre-treatment plants to remove chemical oxygen demand (COD), and other contaminants from their effluents prior to discharge it into receiving waters or publicly owned treatment plants.

Modern and environmentally friendly technologies are incorporated into the production process of textile industry many years ago. Even though, the nature and quantities of the auxiliaries used during textile manufacturing operations have direct effects on the environment, there are not so many published researches about the toxic impact and biodegradability of these chemicals (Park and Shore, 1984; Arslan-Alaton, 2003, 2004). Currently researchers are struggling to set up a complete data inventory about the release of the mentioned chemicals from textile mills to wastewater treatment plants, municipal sewers and receiving water bodies. Therefore, there is a need for dependable and rapid assessment of the environmental effects of textile auxiliaries.

1.2 Aim and Scope of the study

The chemicals used in wet processing stages have mostly xenobiotic in character so textile industry's treatment plants can not treat effluents successfully. However, chemical treatment processes can be applied rather efficiently for the removal of toxic and refractory pollutants (Marco et al., 1997)

Among the refractory pollutants used in textile preparation, dyeing and finishing processes, textile tannins have concerned great attention due to their common usage and particularly refractory character. Synthetic and natural tannins contain poliphenolic and multiple structured oligomeric compounds so they have high molecular weights. Synthetic and natural tannins are frequently used to secure highest levels of wash fastness (particularly to deep depths of shade) and increase the fixation rates during polyamide and nylon acid dyes after rinsing stage (Burkinshaw, 1995).

In recent years, ozone has been used to degrade the unbiodegradable pollutants. Also, ozone used for preoxidation, post treatment and disinfection of domestic and industrial wastewaters (Masten and Davies, 1993).

Among the typically used oxidizing agents in water pollution control, ozone is chosen for tannins' pretreatment as a relatively powerful oxidant with effective performance in low doses (<1-2 mgO_3/mg COD) (EPA, 1999; Hoigné, 1988).

In the above given context the aim of the study is to examine the effect of ozone pretreatment on the biodegradability and toxicity of two commercial; synthetic and natural tannin solutions used in textile dyeing operations. The toxicity was assessed on marine micro algae *Phaeodactylum tricornutum.*

The study consists of the following five chapters.

- In chapter one the importance, the aim and scope of the study were explained.
- In chapter two textile industry's process description, especially dyeing processes were given and the importance of biodegradability based characterization; tannins and their characteristics are explained briefly.
- In chapter three the materials and methods used in the study were summarized. The inert COD test, sequential filtration/ultra filtration test and toxicity tests conducted on marine algae *Phaeodactylum tricornutum* were given in this chapter.
- In chapter four the experimental results were represented and evaluated.
- In chapter five an overall evaluation was performed.

2. LITERATURE SURVEY

2.1 Textile Industry

Production capacity, plentiful raw materials, cheap labour and continuous modernization investments are all factors in Turkey's continuing activity in the textile sector. The industrialization efforts of sixties and seventies gave birth to the modern textile industry in Turkey. Now, the textile preparation, dyeing and finishing industry is one of the most important sectors in the Turkish economy in terms of GDP (Gross Domestic Product), employment and exports (www.igeme.org.tr). According to 2000 Figures, the expected amount of annual cotton yarn production in Turkey was around 788000 tons (www.igeme.org.tr). The share of this textile sector in total industrial production of the country was around 40 % due to the ITKB Figures (ITKB, 2003).

Textile industry gained importance in mid twentieth century and now it is the most important and the largest industry. The variations in materials used, the technique applied and the chemicals added make the textile industry more complex. Textile effluent doesn't have a distinctive nature because of the variety of operations and processes. The quantity and quality of wastewater also differ from each other for separated processes of the same industry (US EPA, 1997c).

Textile industry consists of separated, strictly different processes to manufacture yarn and fabrics into cloths, industrial objects and house upholsteries. To obtain fibers and make ready to process; fibers are converted into yarn and thread used for knitting, weaving, or sewing; is converted into cloth or correlated goods; and dye and finish the supplies at different of processes of manufacture are the steps of textile establishments (US EPA, 1997c).

2.1.1 Textile Industry Processes

The four basic processes in textile industry can be summarized like this as shown in Figure 2.1 (US EPA, 1997c).

a) Yarn production
b) Fabric formation
c) Wet processing
d) Fabrication

2.1.1.1 Yarn production

There are a lot of combining and distorting processes to tie fibers tightly together for transforming them to yarn. Fibers are being used in textile industry can be natural or manmade. Cotton and wool are natural fibers, consist of animal and plant fibers, they require some series of training steps like breaching, combining, carding, brushing, and drafting before whirling into yarn (US EPA, 1997c).

2.1.1.2 Fabric formation

Weaving and knitting are basic processes of fabric formation to generate fabrics. Broadwoven mills, which are a part of weaving mills, generate the raw textile fabric by using the major part of textile fibers. Firstly to use in industrial applications narrow natural fiber, nonwovens and rope are generated. Interspersing one series of yarns with another series crossed diagonally constitute fabrics by weaving. The most frequently used weaving fabrics are satin, sheer and puffy waves (US EPA, 1997c).

Knitting is the next major technique of fabric formation. The adaptability of methods, flexibility of manmade fibers and the increase in consumer demands for wrinkle -resistant, elasticized, comfortable fabrics are increased the usage of knitting as a fabric formation. Fastening one or more series of yarns among bows by using pins is the way to make knitted fabrics. Underwear, suits, coats, carpets are constructed by using knitted fabrics (US EPA, 1997c).

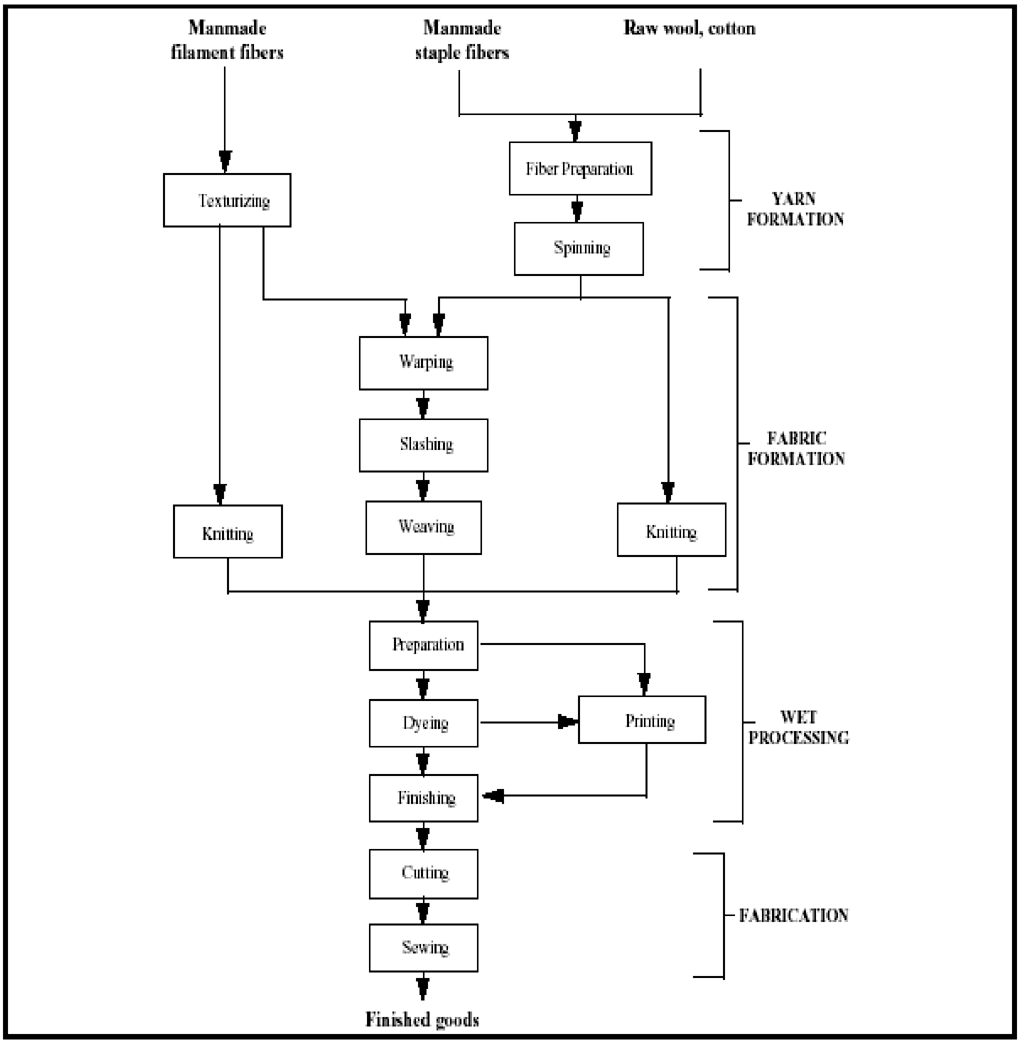


Figure 2.1: The flow chart of basic processes of textile industry (US EPA, 1997c)

The adaptability of methods, flexibility of manmade fibers and the increase in consumer demands for wrinkle-resistant, elasticized, comfortable fabrics are increased the usage of knitting as a fabric formation. Knitted fabrics can be made up by fastening one or more series of yarns among bows with pins. Underwear, suits, coats, carpets are constructed by using knitted fabrics (US EPA, 1997c).

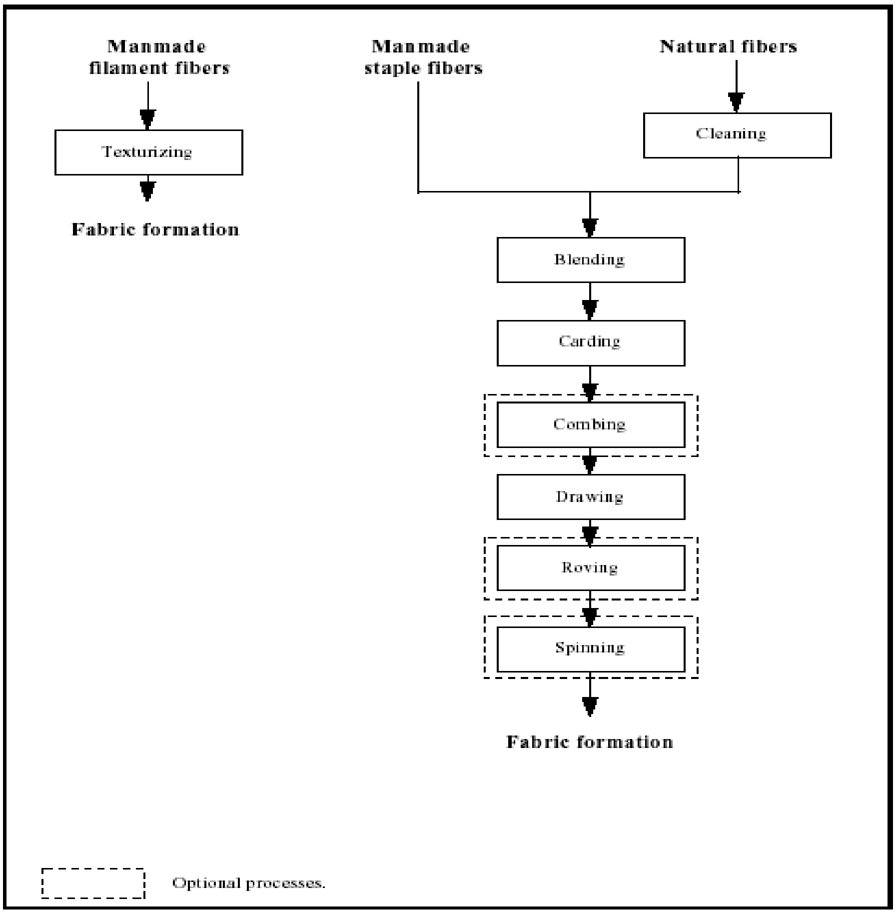


Figure 2.2: Yarn production process (US EPA, 1997c)

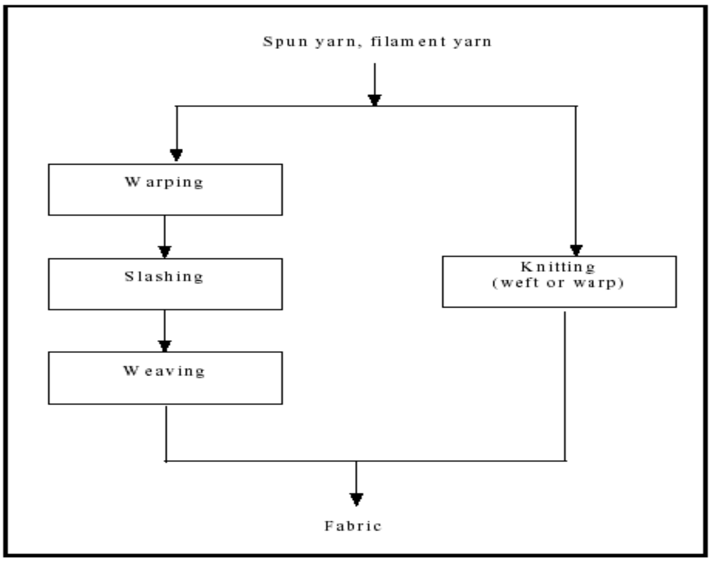


Figure 2.3: General Fabric Formation Processes Used for Producing Flat Fabrics (US EPA, 1997c)

2.1.1.3 Wet Processing

Woven and knit fabrics can not be transformed into apparel and other finishing goods until the fabrics have passed through several intensive wet processing stages. Wet processing enhances the appearance, durability and serviceability of fabrics by converting undyed and finished goods (known as gray or greige goods) into finished customers' goods. Fibers, yarns, thread and woven are also going through dyeing and finishing processes besides knitted fabrics. Wet processing can be investigated in four basic part; fabric preparation, dyeing, printing and finishing (US EPA, 1997c).

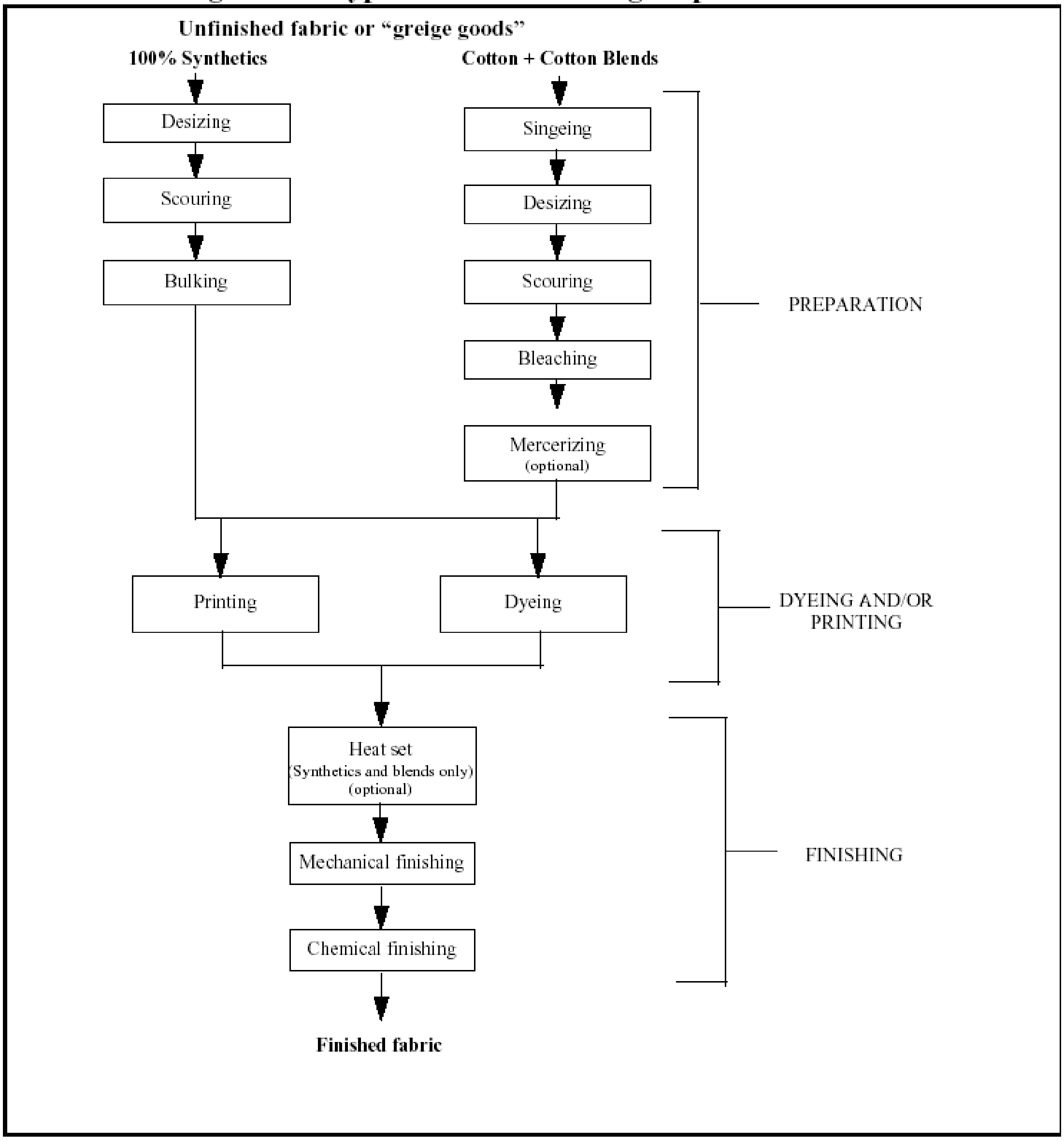


Figure 2.4.: Typical Wet Processing Steps for Synthetic and Cotton Fabrics(ATMI, 1997a)

Preparation is necessary before dyeing and printing processes of fabrics and as well this stage with various rinsing steps is considered as pretreatment, for the best quality in the following textile finishing processes. Textile mill takes place in preparation process takes the dirty materials away and treats chemicals that hinder the dyeing, printing and finishing processes. Fabrics change chemically and physically when going through some preparation steps such as singeing and mercerizing. Natural residuals and the residuals of chemicals are being used in this process make up the waste of preparation process (NC DEHNR, 1986).

2.1.1.4 Dyeing

Dyeing processes are applied to add color and complexity to textiles such as fibers, yarn, woven and thread for increasing the product value. Today, textiles can be dyed with almost any color to achieve a variety of aesthetic and functional purposes. Especially dyehouses are commonly being used in dyeing operations. Wide range of dyestuffs, techniques and equipments are used in dyeing process. Most of dyes being used in textile industry are synthetic which consist of coal and petroleum based intermediary materials (US EPA, 1997c).

Continuous or batch processes can be used as dyeing method.
Batch dyeing; textile substrate between 100 and 1000 kg is being loaded with a dyeing solution into a dyeing reactor. Dyes and fibers have similarity characteristics so they push each other, and in the course of time the dye molecules starts to leave the dye solution and the dyes are beginning to join into fibers, related to the kind of fiber and dye. To organize the dyebath conditions some supplementary chemicals are used for speeding up and balancing the activity. Beam, beck, jet, and jig processing are the most well known batch dyeing processes (US EPA, 1997c).

Continuous dyeing; the range at speeds 50-250m/min is used for loading the textile substrate into dye continuously. In textile industry the usage of continuous dyeing processes is nearly 60 percent of total dyed manufactured goods (Snowden-Swan, 1995). Dye application, dye fixation with chemicals and rinsing are the stages of a typical continuous dyeing process. The dye fixation rate on the fiber processes changes according to the type of dyeing process; in continuous dyeing dye fixation part is much faster than batch dyeing (US EPA, 1997c).

In organization of dyes;. Table 2.1 lists the major dye classes, fixation rates, and the types of fibers. Textile companies control type of fibers, desired shade, dyeing uniformity, and fastness (desired stability or resistance of stock or colorants to influences such as light, alkali, etc) before selecting the kind of dye for application (FFTA, 1991). For cotton dyeing; reactive and direct types; for polyester dyeing; disperse types are being used. Direct dyes are the plainest and the lowest priced dyes (Corbman, 1975). Direct dyes have a fixation rate of 90 to 95 percent and reactive dyes 60 to 90 percent. To increase the dye fixation and absorption into fibers sort of supplementary components can be applied to dyeing operation (US EPA, 1997c).

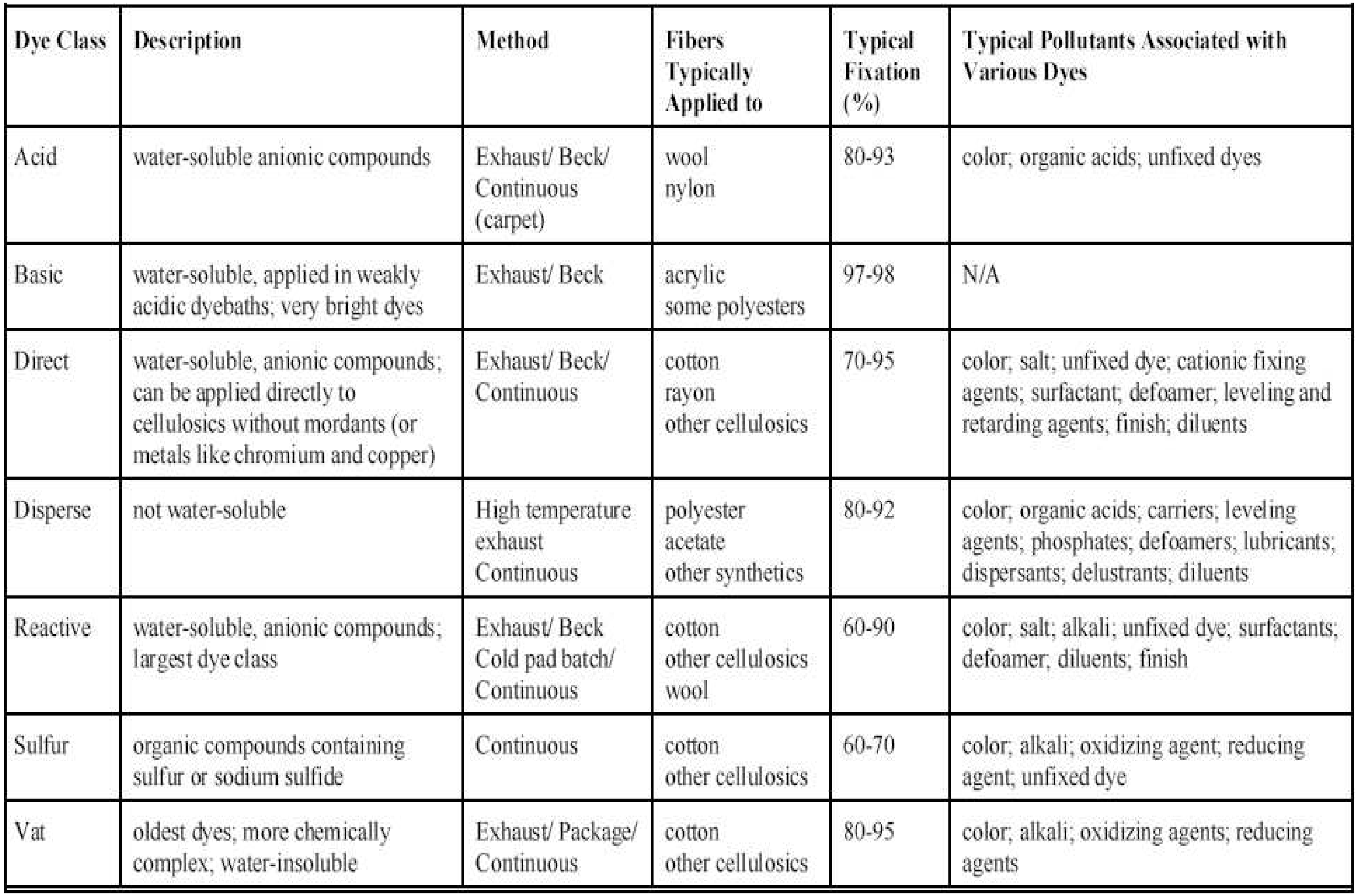
Disperse dyes need extra factors to be used with use synthetic fibers, such as dye carriers, pressure, and temperature (Snowden-Swan, 1995; ATMI, 1997). For cotton and other cellulosic fibers; usually vat dyes are being used as well (US EPA, 1997c).

Most acid dyes are sodium salts of organic sulfonic acids. The acid dyes have a direct affinity towards polyamide and protein fibers in an acidic dyebath (US EPA, 1997c).

2.1.1.5 Printing

Fabrics are frequently printed with dye and modeled using a sort of operation types and mechanisms. The frequently used printing methods are direct, discharge, resist, flat screen, rotary screen and roller printing. Dyeing pigments produce not more residue and do not need washing part although they are applied 75 to 85 percent of all printing techniques (Snowden-Swan, 1995).

Table 2.1.: Typical Characteristics of Dyes Used in Textile Dyeing Operations (US EPA, 1997c)



Dye Class	Description	Method	Fibers Typically Applied to	Typical Fixation (%)	Typical Pollutants Associated with Various Dyes
Acid	water-soluble anionic compounds	Exhaust/ Beck/ Continuous (carpet)	wool nylon	80-93	color; organic acids; unfixed dyes
Basic	water-soluble, applied in weakly acidic dyebaths; very bright dyes	Exhaust/ Beck	acrylic some polyesters	97-98	N/A
Direct	water-soluble, anionic compounds; can be applied directly to cellulosics without mordants (or metals like chromium and copper)	Exhaust/ Beck/ Continuous	cotton rayon other cellulosics	70-95	color; salt; unfixed dye; cationic fixing agents; surfactant; defoamer; leveling and retarding agents; finish; diluents
Disperse	not water-soluble	High temperature exhaust Continuous	polyester acetate other synthetics	80-92	color; organic acids; carriers; leveling agents; phosphates; defoamers; lubricants; dispersants; delustrants; diluents
Reactive	water-soluble, anionic compounds; largest dye class	Exhaust/ Beck Cold pad batch/ Continuous	cotton other cellulosics wool	60-90	color; salt; alkali; unfixed dye; surfactants; defoamer; diluents; finish
Sulfur	organic compounds containing sulfur or sodium sulfide	Continuous	cotton other cellulosics	60-70	color; alkali; oxidizing agent; reducing agent; unfixed dye
Vat	oldest dyes; more chemically complex; water-insoluble	Exhaust/ Package/ Continuous	cotton other cellulosics	80-95	color; alkali; oxidizing agents; reducing agents

2.1.1.6 Finishing

Finishing processes contain chemical and mechanical operations to change characteristic or strength of yarn, fiber and fabric for increasing the view.

Mechanical operations such as brushing and ironing are being applied to improve shine and sense of textiles. Permanent-press, soil release and stain-resistant finishes are being used to facilitate the fabric attention (Snowden-Swan, 1995).

2.1.1.7 Fabrication

Fabrication step is being used to transform finished fabric into a variety of clothing, household and industrial goods. Cutting trades is most frequently used process as fabrication for clothings and more complex house wares such as bags, sheets, towels, blankets, and draperies. Finished fabrics must be attentively arranged out because if any mistake made this part of operation it affects the other processes and also the final good (US EPA, 1997c).

2.1.2. Process and Pollution Profiles of Textile Industry

Textile industry is the most polluting industry among all industries when the effluent characteristics are considered. The wastewater of textile industry includes cleaning water, process water, non contact cooling water, and storm water. The amount of water used changes widely in the industry, depending on the specific processes operated at the mill, the equipment used, and the established management philosophy concerning water use. Because of the wide variety of processing steps, textile wastewater typically contains a complex mixture of chemicals (US EPA, 1997c).

Therefore, from the environmental point of view, the textile industry is also one of the most water and chemical intensive industries worldwide (Correia et al., 1994). Aqueous effluent from textile mills has been identified as the primary pollution source of this industry (US EPA, 1997a).

As recently has been reported in the scientific literature, 20–230 m^3 of water is being consumed to produce 1 ton of textile fabric in Turkish factories (Orhon et al., 2003). The organic loads from textile effluents are being ranged between 2.5 – 800kg COD per ton of fabric which corresponds to a range of 15- 5000mg/l COD (Germirli et al., 1998).

Textile wastewater includes aqueous discharges from fiber and fabric preparation, de-sizing, scouring, bleaching, dyeing, finishing and other textile-processing stages. Most mills operate their own wastewater treatment or pre-treatment plants to remove chemical oxygen demand (COD), biochemical oxygen demand (BOD) and other contaminants from effluent. Also they are being used to be prior to discharge into receiving waters or publicly owned treatment works.

Potential releases from the textile preparation, dyeing and finishing process stages are summarized in Table 2.2.

Table 2.2.: Potential Releases emitted during Textile Manufacturing Processes (US EPA, 1997c)

Process	**Wastewater**
Fiber preparation	Little or no wastewater generated
Yarn spinning	Little or no wastewater generated
Sizing	BOD; COD; metals; cleaning waste, size
Weaving	Little or no wastewater generated
Desizing	BOD from water-soluble sizes; synthetic size; lubricants; biocides; anti- static compounds
Scouring	disinfectants and insecticide residues; NaOH; detergents, fats; oils; pectin; wax; knitting lubricants; spin finishes; spent solvents
Bleaching	hydrogen peroxide, sodium silicate or organic stabilizer; high pH
Singeing	Little or no wastewater generated
Mercerizing	high pH; NaOH
Heat setting	Little or no wastewater generated
Dyeing	metals; salt; surfactants; toxics; organic processing assistants; cationic materials; color; BOD; COD; sulfide; acidity/alkalinity; spent solvents
Printing	suspended solids; urea; solvents; color; metals; heat; BOD; foam
Finishing	BOD; COD; suspended solids; toxics; spent solvents
Product Fabrication	Little or no wastewater generated

As showed in the Table 2.2, there are a lot of additives in dyeing process, so it makes this processes' wastewater the most complex. Processing methods may also be different based on the final properties desired, such as tensile strength, uniformity, and elasticity (Snowden-Swan, 1995).

2.2 Auxiliaries used in textile industry and their environmental impacts

According to the use of great variety of fibers, dyes, process additives and finishing products, wet processing in textile industry generate wastewaters that have a high volume with complex nature. Dyeing process effluents can not sufficiently be treated in conventional biological wastewater treatment plant (Vandevivere et al., 1998).

Textile dyeing is the most water consuming and chemically concentrated process among all textile preparation, dyeing, washing and finishing stages. Textile plants usually use cotton, synthetic fibers; and include integrated printing and dyeing operations with application of a wide variety of organic dyes (Weltrowski et al., 1996; Achwal, 1997). Apart from water, auxiliary formulations are being used frequently in textile finishing factories for dyeing baths. Auxiliaries are commonly being used as essential components in dyeing processes. Some of them are biodegradable, while others have poor biodegradability. They are thrifty soluble in water and largely eliminated by adsorption on the activated sludge in wastewater treatment plants (Weltrowski et al., 1996).

Textile dyeing effluent contains residuals of dye bath auxiliaries and unfixed dyestuff at significant concentrations (Grau P., 1991). Unfortunately, most chemicals are being used for textile dyeing are known as having recalcitrant character. Recalcitrant or refractory compounds are hardly biodegradable and also can defend against most physicochemical methods such as adsorption, coagulation or precipitation. They leave the biological treatment unchanged. Due to their existence sometimes it is not possible to meet the discharge standards; and also they threaten the aquatic life in receiving waters (Ince, et al., 1997; Alvares et al., 2001).

The degree of recalcitrance is related to molecular complexity; branching, polymerism. Microbial biodegradation is based on substrate specific enzyme activity, so small structural differences can be reason of recalcitrance (Alvares et al., 2001).

Soluble substances in water are frequently encountered in dyeing auxiliaries. The compounds most commonly used to assist dyeing processes based on their function in the process can be considered as follows: (Rieger et al., 2002)

- De-aerating and penetrating agents: The de-aerating agents are being used to expel the air from textile enclosed in the dyebath. The function of penetrating agents is associated with the dyeing of yarns, where they enhance transport of the dye into the yarn assembly. Frequently used commercial products are largely readily bio-eliminable compounds such as esters, alcohol and polyglycol ethers.
- Dispersing agents: Dispersing agents tolerate the application of colorants in the form of aqueous dispersions. Extra amounts of dispersants are usually being added in the subsequent steps of the dyeing process to maintain the stability of the dispersion during the dyeing process. Dispersing agents are present in the formulation of vat, sulphur and disperse dyes.
- Levelling agents: Levelling agents are being used to improve the uniform distribution of the dye in the fibre. These agents can be grouped into two as products; which have affinity for the fibre and which have affinity for the dye.
- Acid donors: The shift of pH occurs as the acid released by hydrolysis during heating or as one of the acid/base components is transferred to another phase such as the fibre or air, e.g. ammonia release to air with ammonium sulphate. Acid donors represent products designed to create shifts in dyebath pH. They are commonly used for wool and/or polyamide fibres to control the absorption of anionic dye onto the fibre.
- Antifoaming agents: Antifoaming agents are being used for suppressing foam formation. These agents do not affect the dyeing quality negatively. Silicone derivatives are the major group of antifoaming agents.

Additionally, because of the existence of refractory organic macromolecules such as tannins in textile effluent, biological treatment and bioremediation technologies can not be successful to treat these wastewaters (Burkinshaw and Bahojb-Allafan, 2004; Saroj et al., 2005). Since the chemical process can wipe inhibitory compounds out and alter the chemical structure of refractory compounds by means of developing the biodegradability, they have operating cost of ten times more than biological processes. Waste component greatly or totally resistant to usual biological treatment can be completely mineralized using a collective chemical-biological system therefore less oxidant is required for complete chemical mineralization (Alvares et al., 2001).

It is expected that comprehensive studies related with to ecotoxicological properties (biodegradability, bioaccumulation, short-term or chronic toxicity) of textile chemicals will develop new products appearing on the market to meet new environmental regulations concerning the discharge of textile effluent (Alaton et al., 2005).

2.2.1. Tannins

Tannins are natural organic pollutants as polar phenolic polymeric compounds. Their molecular weight changes between 500 and 3000g mol/l (White, 1957). There are two main categories of tannins: hydrolysable tannins and condensed tannins. Except very high weighed compounds; tannins are usually soluble in water (Burkinshaw et al., 2002).

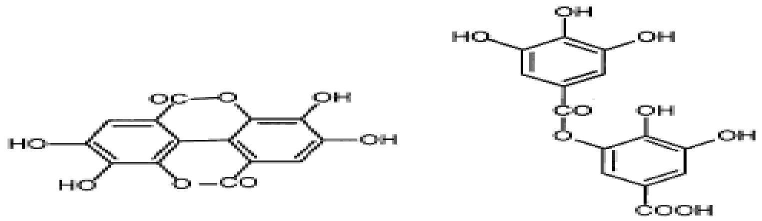
Hydrolysable tannins:

Hydrolysable tannins can react easily with other substances such as gallic acid (-gallotannins) or ellagic acid (-ellagitannins) due to the readily soluble character. Hydrolysable tannins are derivatives of gallic acid (3,4,5 –trihydroxyl benzoic acid). Their structures are shown in Figure2.5.

Condensed Tannins:

Condensed tannins can be named as Proanthocyanidis. They have a condensed chemical nature, even though they are still capable of undergoing further condensation reactions. Condensed tannins contain flavonoid units, with alterations on the sites so the flavan bonds are formed to generate a complex structure. Condensed tannins have rigid carbon–carbon bonds that cannot be broken down simply by hydrolysis. (Pizzi A, 1983.) The most widely studied condensed tannins are based on Figure 2.6.

(a): Pyrogallol (b): Gallic acid



(c): Ellagic acid (d): *m*-Digallic acid

Figure 2.5.: The chemical structures of some phenols synthesized from HT (Bisanda et al., 2003)

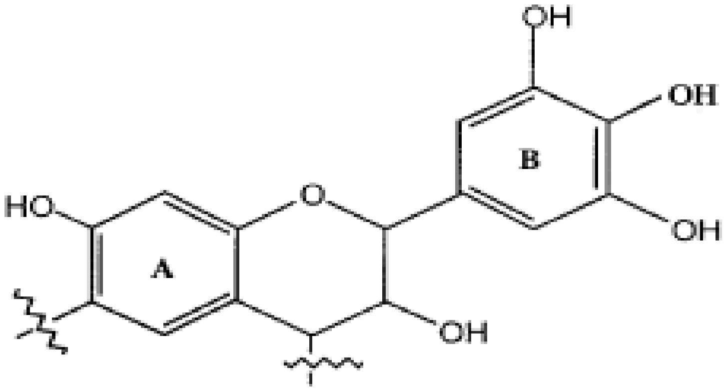


Figure 2.6.: The chemical structure of wattle tannin (Acacia mearnsii) flavonoids.

(Bisanda et al., 2003)

Tannins have found a wide range of other industrial applications; besides their common application in textile industry. They can be used in tanneries, in pulp and paper industry, food beverages, anti corrosion applications, ink manufacturing and pharmaceutical industries (Svitelska et al., 2004).

Tannic acid has a natural source. Vascular plants synthesize large amounts of tannin. Although, tannic acid is a natural compound and found at low concentrations in textile effluent, it is very toxic (Svitelska et al., 2004).

Tannins can originate significant aesthetic troubles and can affect harmfully flora and fauna of recipient's water bodies. It was established that condensed tannins from spruce bark are toxic not only to methanogens at concentrations present in the paper mill effluents (Field et al., 1988), but also to aquatic organisms, like fish (Temmink et al., 1989).

2.2.1.1 Usage of Tannins in Textile Industry

Recently, the dyed goods' fastness through washing, chlorine bleaching besides staining has turn out to be more and more important to answering the increased consumer demands. Among the xenobiotics originating from the textile preparation, dyeing and finishing stages, textile tannins attracted interest because of their common use and refractory structure (Burkinshaw et al., 2003).

Acid dyes are used for dyeing mainly nylon fibers, as well as wool, silk and modified acrylics. It is well known that the wash fastness of acid dyes on nylon 6,6 leaves much to be desired and that 1:2 pre-metallised acid dyes generally display higher levels of wash fastness than their non-metallised counterparts on the substrate (Burkinshaw et al., 2003).

A synthetic or a natural tanning is commonly used to secure highest levels of wash fastness (Burkinshaw, 1995). Due to the chlorine scavenging properties of tannic acid's chemistry, it has defensive action preventing dye stuffs' oxidative degradation. Tannic acids not only protect the textile materials from the attack of bleaching water, but also protect them against oxidizing agents such as H_2O_2. In Figure 2.7. it can be easily observed that both the tannic acid and dye pigment are anionic and their negative character causes an electrostatic interaction between them (Burkinshaw et al., 2003).

Tannic acid

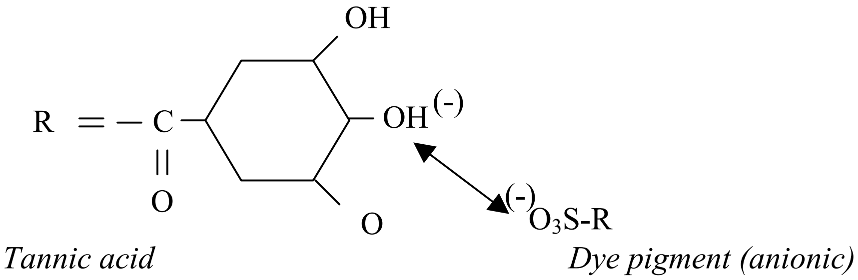


Figure 2.7.: The Interaction between Tannic Acid and Anionic Dyes

Tannic acid can increase the wash fastness and prevent desorption during dyeing process because of the electrostatic pushing force between hydroxyl group of tannin and dyes pigment. In the Figure 2.8., the reaction on nylon dyeing process between nylon and tannic acid is shown.

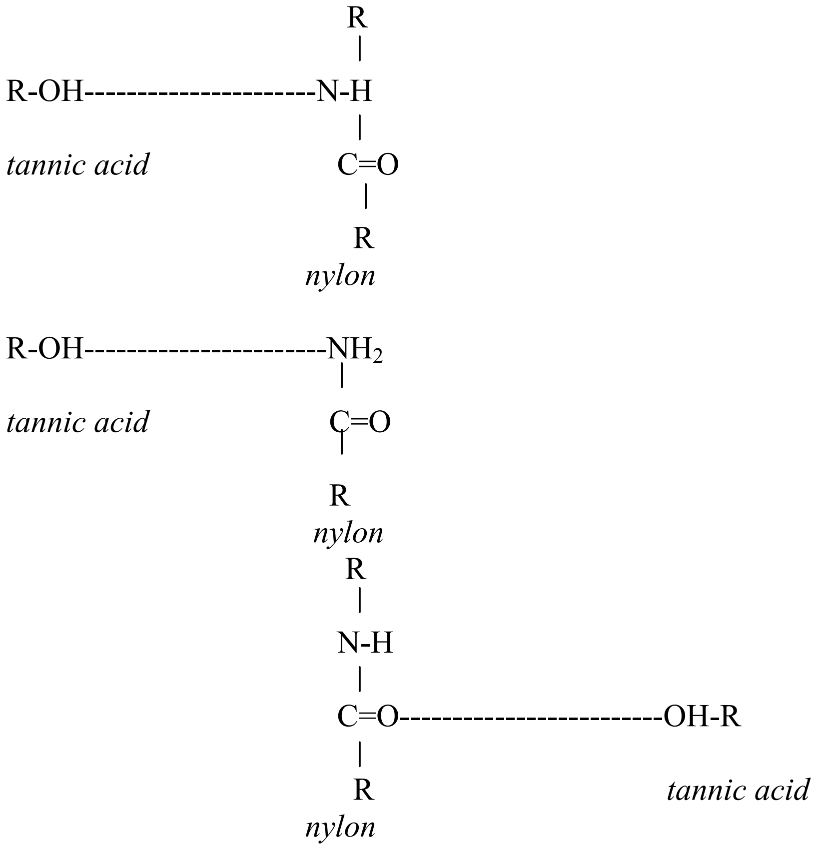


Figure 2.8.: The Electrostatic Interaction between Tannic Acid and Polyamide

In Figure 2.9., the nylon process with and without tannic acid exhibited. The same as the Figure shows, tannic acid molecules increase the fixation rates of anionic dyes. When tannic acid not applied, dye molecules wait on the positively charged polyamide fabric’s outside. While fixation rate of the dye molecules to the fabric increases, the unfixed dye quantity decreases. Moreover the efficiency of dyeing process causes low dye molecules in dyeing effluent.

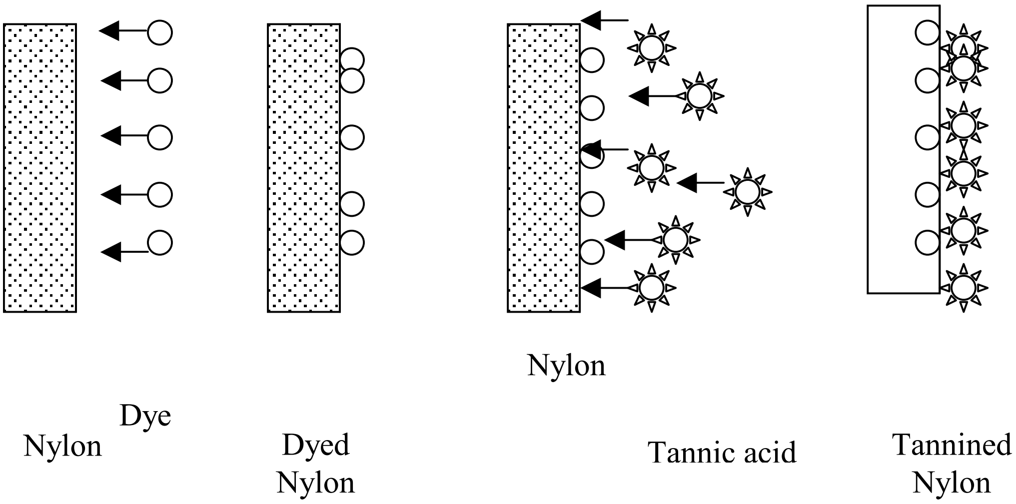


Figure 2.9.: The Comparison of Polyamide Dyeing with and without Tannic Acid

2.2.1.2 Treatability and Toxicity of Tannins

Biological treatment is one of the most widespread methods, applied for the treatment of textile industry mills effluents. Though, tannins were found to be toxic for numerous enzymes (Gupta and Haslam, 1980; Korczak et al., 1991; Sierra-Alvarez et al., 1994). The hydrogen-bonding reactions with proteins create toxicity towards bacteria, because such interactions hinder with the performance of enzymes (Gupta and Haslam, 1980). They are also known to exhibit methanogenic toxicity depending on the level of polymerisation (Field et al., 1988). During the aerobic wastewater treatment tannins undergo more polymerization due to intensive aeration, forming colored high molecular weight compounds.

Tannins can be also a reason of serious aesthetic problems and influence negatively flora and fauna of receiving water bodies. Also tannins cause turbidity in wastewater, which have a tendency to absorb more neat, light and keep less oxygen. As a result of this property, an extra treatment step for the removal of tannins from overall wastewaters is desirable (Svitelskaet al., 2004).

2.3. Biodegradability Based Characterization

A conventional active sludge system, which has two components; a substrate and biomass, has been lost the validity. In the frequently used conventional active sludge systems the effluent wastewater quality is assumed to be independent from influent wastewater quality and studies have gone through with the opinion of effluent wastewater has the same character with substrate. Optimal design of biological treatment for wastewaters now requires a mathematical modelling to evaluate the system behavior. Such models can be reliable if only they are structured upon meaningful process components of substrate and biomass.

Activated sludge modeling is connected with the consumption of COD because this parameter indicates the electron equivalence between organic substrate, active biomass and dissolved oxygen. The main requirement of modeling is a reliable wastewater characterization with COD fractionation, for the identification of organics with different biodegradation properties. In this respect, assessment of the inert fraction of the organic content of the wastewater is very important because it indicates indirectly the other main fraction, biodegradable organic matter, i.e. electron acceptor utilization and substrate available for microbial growth. Inert COD component may be of influent origin or they may be generated as residual microbial products by means of growth or decay-associated processes (Daigger & Grady, 1977; Orhon et al., 1989). Several methods have so far been proposed for the estimation of inert COD fractions in wastewaters (Orhon et al., 1999). Later, Orhon et al. (1994a) proposed another procedure involving monitoring of the particulate COD in a batch reactor, both accounting for soluble and particulate inert products. The study covers identification of COD fractions in terms of their biodegradation characteristics. COD fractionation is used because;

- ✓ BOD_5 and total COD are unaccepTable as substrate parameters in process kinetics
- ✓ They should not be used as process components (model components)
- ✓ COD reflects a large spectrum of organic compounds with different biodegradation characteristics
- ✓ A single rate expression, using overall (total) COD as the only component, is totally misleading
- ✓ Expressing COD in terms of different COD fractions with markedly different biodegradation properties

The overall or total COD, although a convenient parameter, cannot be regarded as a valid process component in recent models for carbon and nutrient removal. Because it covers, as illustrated in Figure 2.10., a large spectrum of organic compounds with different biodegradation characteristics with inert components of influent origin or generated during biological treatment as residual microbial products. Consequently, wastewater characterization, if conceived as a useful tool to supply the necessary information for process modelling, should include COD fractionation as well as the assessment of conventional polluting parameters.

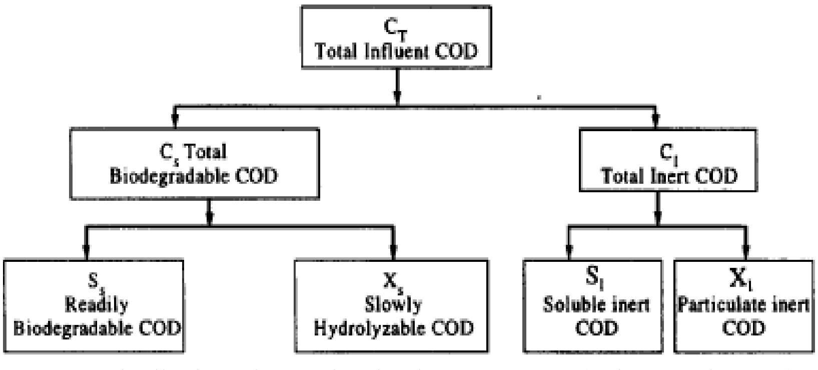
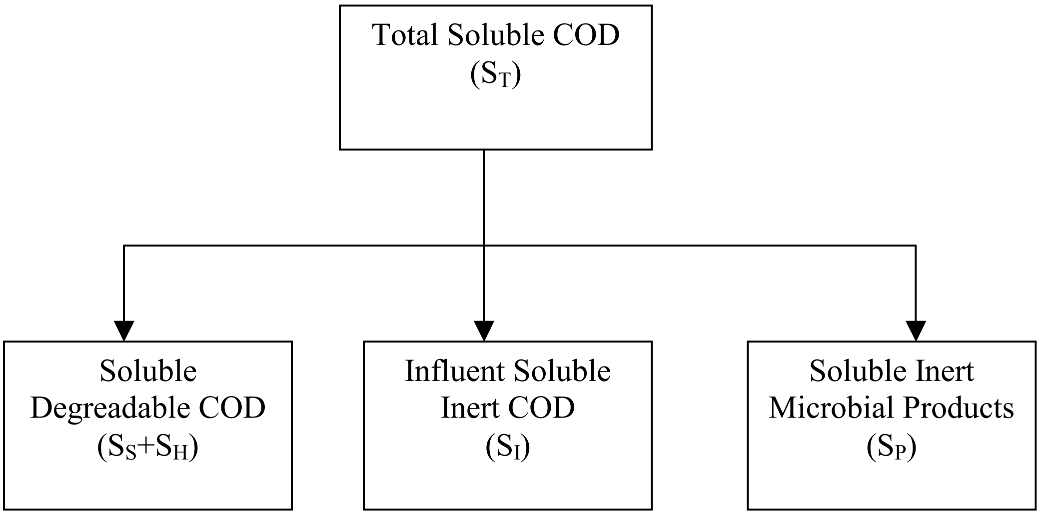


Figure 2.10.: Distribution of COD fraction in wastewaters (Orhon, et al., 1994)

The commonly adopted approach today is to evaluate the total COD (C_T) as two major components of biodegradable COD (Cs) and non biodegradable or inert COD (C_I). Experimental evidence also indicates that the entire COD content of wastewaters cannot be removed through biological degradation, due to the presence of non-biodegradable or inert COD (C_I). The inert fraction is analyzed in two subgroups of soluble inert COD (S_I) and particulate inert COD (X_I). Soluble inert COD goes through treatment plant and remains unchanged in the effluent. However, particulate inert COD remains in the biomass and disposed with sludge removal plant after settlement (Orhon et al., 1996).

Two major components of the total biodegradable COD (Cs) are readily biodegradable COD (Ss) and slowly biodegradable COD (Xs) in accordance with the original bi-substrate model proposed by Dold et al. (1980). Obviously, both components are likely to include a number of organics with a variety of biodegradation rates, but this range is of no practical importance when compared with the rates separating these two groups (Dold & Marais, 1986). Slowly biodegradable group is generally observed to cover a large number of compounds of different size and nature. The readily biodegradable COD can be directly absorbed for synthesis, whereas hydrolysis is required first for the utilization of the slowly biodegradable COD. Hydrolysis is known as rate limiting step due to the usage of slowly biodegradable organic matters. It is hard to characterize hydrolysis step because it differ for various organic matters in wastewater. Thus, with a new approach slowly biodegradable COD was subdivided into further fractions, namely rapidly hydrolysable COD (S_H) and slowly hydrolysable COD (Xs) (Henze et al., 1987)



(a) Soluble COD Fractions

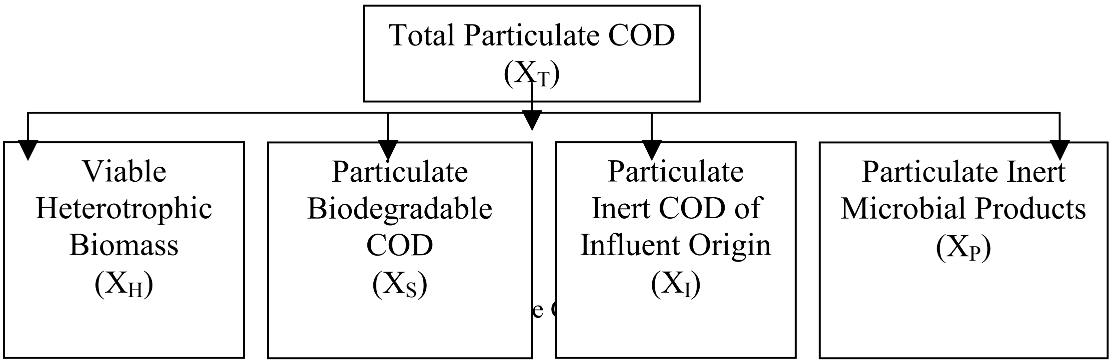


Figure 2.11. Distribution of COD fraction in mixed liquor and effluent (Orhon, et al., 1994)

The mixed liquor and the process effluent exhibit a different structure than wastewater. Total soluble COD (S_T), includes the nonbiodegradable organics originating from the wastewater and bypassing the reactor, S_I, a small portion of the biodegradable COD remaining after biological oxidation, $S_S + S_H$, and soluble residual COD generated as metabolic products, S_P. Consequently the effluent stream includes more soluble inert COD than influent wastewater (Orhon et al., 1989).

Similarly, the particulate COD in the mixed liquor and the process effluent as four fractions as showed in the Figure 2.11.. The major component is the active biomass, X_H, that uses the biodegradable COD as energy and carbon source to sustain in the reactor. The other component is a small portion of the particulate slowly hydrolysable COD, Xs, remaining after hydrolysis and subsequent utilization. The mixed liquid also includes particulate inert COD of wastewater origin, X_I, kept in biomass the biomass and accumulated in the reactor. There is also another particulate nonbiodegradable fraction; particulate inert organic products, X_P, are generated as a result of the microbial metabolic activity during endogenous decay.

2.4. Toxicity Testing

In natural aquatic environments there are various organic and inorganic materials which are coming from several sources such as atmosphere, surface run-off etc. The effects of those chemicals on microorganisms can not be evaluated only with chemical analyses (Okay, 2005). Chemical and physical measurements are used to determine the concentration of the toxicant and how the environmental quality affected is determined by toxicity tests. Chemical concentration can be measured with an instrument, but toxicity can be measured only with usage of a living material (Cairns, et al., 1990).

Toxicity tests concern with the relationship between quality and quantity of chemical that expose an organism; and the nature and degree of toxic effects.

Most toxicity tests have evaluated the short-term lethality of wastes/chemicals to adult fish. Fish have been the most popular test organism because it has been thought to be the most understandable organism in aquatic environment. However, when discharging area is small, a wastewater entering into an aquatic ecosystem may influence thousands of species. Previous studies (Patrick, 1949; Patrick 1968; and others have shown that diatoms and macro invertebrates are often more sensitive to toxicants than fish, and represent a greater majority of the biomass in a natural system than do fish. As well, fishes can commonly use algae and macro invertebrates as food. Even if the aim is protection of fish alone, it requires testing and protecting other members of the communities on the environment. As a result of this reality, usage of other organisms such as algae, aquatic plants, and invertebrates have become growingly popular and common in toxicity tests rather than fish.

There are various rules for selecting toxicity test species simply it is based on four criteria:

- The organisms are representative of an ecologically important group (in terms of taxonomy, trophic level or realized niche).
- The organism occupies a position within a food chain leading to a man or the other species.

- The organism is widely available, is amenable to laboratory testing, easily mentioned, and genetically Table so uniform populations can be tested.
- There is adequate background data on the organism (i.e. its physiology, genetics, taxonomy, role in natural environment must be well understood).

Consequently, toxicity test with microalgae are frequently used to evaluate the affect of potential contaminants that may enter environment (Rand, 1995).

Algae show several responses to toxicant such as growth inhibition and simulation or morphologic and physiological changes. The frequently reported impact is growth inhibition based on changes in biomass. Algal toxicity test is simple to conduct and relatively inexpensive if compared with others (Elnabawary and Welter, 1984; Nyholm and Kallqvist, 1989; Thursby et al). Algal grow fast and if not killed by a short term exposure during transitory episodic events can recover quickly. A variety of statistical techniques have been used to analyze algal toxicity data.

Acute tests were considered ecologically significant, most scientifically and legally defensible, most simple and cost effective, and have the greatest effectiveness (Buikema, 1982). The value of acute toxicity test data can be maximized by careful consideration of the purpose of the experiment, utilizing the appropriate design, and reporting relevant factors besides mortality. Data may be expressed as an effective concentration (EC). The EC_{50} value is the statistically derived concentration of test substance in an environmental medium expected to produce a certain effect in 50% of test organisms in a given population under a defined set of conditions (Walker et al., 2001). Most published methods recommend reporting the EC_{50} values (Buikema, 1982).

3. MATERIALS AND METHODS

3.1. Materials

The samples were prepared to simulate natural and synthetic tannin added dyebath discharges in batch dyeing processes. The experiments were applied on raw and ozone-pretreated natural and synthetic tannin. Raw natural tannin (NT) and raw synthetic tannin (ST) solutions prepared as indicated below were used as discharges countaining refractory and toxic pollutants arising from dyeing and finishing stages of a local textile mill. Pretreated natural tannin (pNT) and preatreated synthetic tannin (pST) solutions were used to examine if pretreatment is supportive for decreasing the refractory nature and toxicity of tannin solutions.
Characteristics of NT and ST are given in Table 3.1.

Table 3.1.: Physicochemical Properties of the Selected Textile Tannins, ST and NT (Safety Data Sheet Information).

Property	**ST**	**NT**
Appearance	Yellow liquid with a characteristic odour	Brownish liquid With a weak odour
Ingredients	Aqueous solution of tannic acid	A methylene linked condensation product of arylsulphonic acids and hydroxyaryisulphone preparations
Density (g/cm^3)	1.15 at 25°C	1.10 at 23°C
pH	2.5-3.5	7.0-8.0
Viscosity (mPa.s)	10-100at25°C	< 50 at 23°C
Solubility	Highly water soluble	Highly water soluble
Acute oral toxicity (LD$_{50}$;*mg/g)*	>2000	>2000
Fish toxicity (EC$_{50}$; mg/L)	>100**	1 -10***

*Lethal dose causing 50 % death in rats;
**Lethal concentration of pollutant (in mg/L) causing 50% death of the studied test Organism, Zebra fish
***Lethal concentration of pollutant (in mg/L) causing 50% death of the studied test Organism, *Leuciscus idus*

3.1.1. Natural Tannin

In textile mill NT is used in acid Nylon 6, 6 batch dyeing process at a pH value of 3-4. The textile mill's water consumption is taken as 2400l and weight of fabric produced is approximately 300kg. The NT is applied at a ratio that corresponds to

5% of cloths weight and 25% NT remains unfixed in the waste of dye bath where tannin is added.

Therefore according to the simple calculation given below
300kg cloth*(5%)/cloth* (25%)/ 2400l

The experiments were performed on the NT samples prepared by mixing 1.56 g NT to a liter of distilled water in order to obtain simulate batch dyeing processes.

3.1.1.2. Synthetic Tannin

In textile mill ST is used in batch dyeing process at a pH value of 3-4.
The textile mill's water consumption is taken as 2400l and weight of fabrics produced is near 300kg.
The ST is applied 4% of cloths weight and 25% ST remains unfixed in the waste of dye bath where tannin is added.

Therefore according to the simple calculation given below
300kg cloth*(4%)/cloth* (25%)/ 2400l

The experiments were performed on the ST samples prepared by mixing 1.25 g ST to a liter of distilled water in order to simulate batch dyeing processes.

3.1.2. Pretreated Materials

Due to toxic character and low biodegradability pretreatment is required before discharging the wastewater that contains tannin into both the treatment plant and the receiving water. According to the results of a previous study the optimum outputs can be obtained by applying ozone as pretreatment. (Koyunluoğlu, 2005)NT and ST solutions pretreated with ozone at an ozone feeding rate of 1000mg/h at pH of 3.5 for 40 minutes are used in the experiments.

3.2. Analytical Methods

3.2.1. Conventional Parameters

All analyses were performed as defined in Standard Methods (APHA, 1989). The soluble (filtered) COD was defined as the filtrate through Whatman GF/C glass fibre filters which were also used to assess SS and VSS parameters.

3.2.2. Determination of Inert COD

Determination of inert COD fractions became more important than determination of the kinetics of biodegradable part of influent COD in recent studies. (Germirli et al., 1993) The effluent COD of the biological treatment plants does not include only the residual part of the influent organic matter but also it contains the residual metabolic products formed in biological processes. The inert COD concentrations are very important to meet the discharge standards.

An experimental method developed under aerobic conditions for the direct measurement of the influent inert COD fractions (Germirli et al., 1993a). This procedure can be applicable only wastewaters that do not include particulate matters.

In this thesis samples (NT&ST) have soluble characteristic. Consequently, this experiment requires two aerobic batch reactors; first reactor was fed with soluble wastewater and second reactor feed with glucose. The biomass used in the reactors is acclimated at an F/M ratio of 0.7 before this experiment. For acclimation period four fill and draw reactors were used first two was feed with 50% glucose and 50% raw material (NT and ST), the other two feed with 50% pretreated material (NT and ST) and 50% glucose. Solution A was used for nutrients, nitrogen and phosphorus requirements; Solution B was used trace elements to supply of Mg, Mn, Fe, Zn and Ca. The application quantity for nutrient and buffer supplies is 10 ml solution A and solution B / 1000mg/l COD.

Prior to the start of this experimental procedure, the biomass taken from the aeration tank of the corresponding treatment facility, was acclimated to a mixture of the biological treatment influent and glucose solution, with a 1:1 ratio in terms of COD content. Then portions of this acclimated biomass (50mgVSSL/1) were used as seeds for two aerated batch reactors, one fed with filtered wastewater, and the other with glucose solution having a COD equivalent to that of filtered wastewater. The test was conducted for a long time to enable the depletion of all biodegradable organics and mineralization of biomass. Residual COD values of the two batch reactors were used to calculate S_I.

The experiments were performed at room temperature, at a pH range of 7-8. Buffer and nutrient solutions were used for elimination of probable pH variations and nutrient limitations during the experiment. Eight one liter reactors were used for this experiment.

After monitoring of COD measurements of each reactor for a long period; a steady threshold soluble COD was obtained. Each reactor was run until all biological activity was finished and no significant COD alteration was observed. The threshold soluble COD value covers; the biomass decay processes' residual products and the inert soluble COD of influent wastewater (Dulekgurgen, et al., 2006).

Y_{SP} is the ratio of the residual soluble per biodegradable COD of influent wastewater microbial end products generated in the reactor. The experimental procedure depends on the concept that Y_{SP} calculated in the glucose reactor is equal to the Y_{SP} in the wastewater reactor.

$$Y_{SP} = \frac{S_{GP}}{S_{GO}} \tag{3.1}$$

$$Y_{SP} \cdot S_{S0} + S_I = S_{T0} \tag{3.2}$$

$$S_T = S_{S0} + S_I \tag{3.3}$$

From equation 3.1 and 3.2 the following equation can be obtained

$$S_T - S_I = Y_{SP} \cdot (S_{T0} - S_I) \tag{3.6}$$

where

S_{PG} : residual soluble microbial end products generated in glucose reactor (mg COD/l)
S_{G0} : initial soluble COD of glucose reactor (mg COD /l)
S_{T0} : initial soluble COD of wastewater reactor (mg COD /l)
S_{S0} : initial soluble biodegradable COD of wastewater reactor (mg COD /l)
S_T : final soluble COD of wastewater reactor (mg COD /l)
S_I : initial soluble inert COD of wastewater reactor (mg COD /l)

Y_{SP} : ratio between the soluble residual products and biodegradable COD

In the present study inert COD determination method applied to find out the fractions of the NT and ST solutions and to observe the effect of ozone pretreatment on the bio-treatability.

3.2.3. Sequential Filtration/Ultra filtration Experiments

In wastewaters particles can be categorized into size groups, namely dissolved (<1nm), colloidal (1–103 nm), supracolloidal (103–105 nm) and settleable (>105 nm). In recent times, ultrafiltration, is effectively applied to recognize and separate wastewater pollutants within much narrower ranges (Engström and Gytel, 2000; Sophonsiri and Morgenroth, 2004; Doğruel et al., 2006).

Differentiation of various COD fractions in terms of their molecular weight cut-offs was determined and also the effect of chemical treatability on these fractions was investigated.

Experimental Procedure;

- Amicon, 8400 Model 400ml volume cell experiment apparatus used with a mixer as shown in Figure 3.1
- All ultra filtration experiments were applied under positive pressure (0.6–1.2 atm; N_2 as the inert gas)

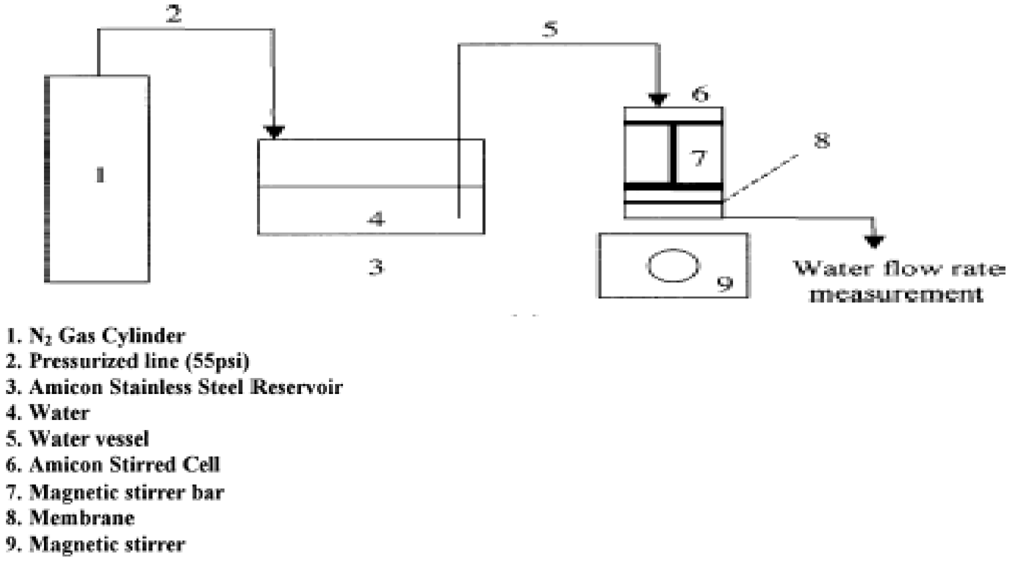
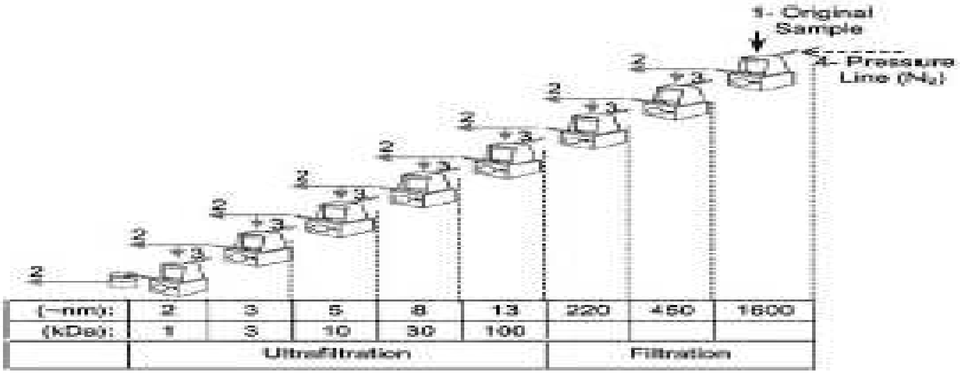


Figure 3.1 Schematic illustration of water flux measurement of the membrane.: .

Firstly samples were filtered through conventional filters consecutively with pore sizes of 1200–1600nm (Millipore AP40, glass fiber), 450nm (Durapores HV, polyvinylid fluoride [PVDF]) and 220nm (Durapores GV, PVDF) (Millipore Corp., Bedford, MA 01730).

- The working pressure was kept at 0.35atm throughout filtration experiments with disposable filters.
- Leakage coming from 220nm membrane filter was filtered successively through ultra filtration membranes with nominal molecular weight cut-off (MWCO) values of 100, 30, 10, 3, and 1 kDa (PL series, Millipore, MA).

- Recommended maximum running pressure was 0.7 atm for the 100kDa membrane, and 3.7 atm for the other ultra filtration membranes
- All experiments were carried out at room temperature (20^0 C) and at a pH range of 7.2–8.8
- A volume of 100ml permeate was collected with the subsequent filtration/ultra filtration steps to run COD experiments in duplicates
- Filtration/ultra filtration was performed as batches of 10–45,minutes, depending on the filter pore size
- All cleaning (repeated washes with distilled water, 70% ethanol, and finally two times with distilled water, under the selected working pressure, as well as rinsing the complete system with distilled water after washing with 70% ethanol), testing (with distilled water), and distilled conditioning procedures, were performed before and after filtering the samples, to make certain that no residue of fouling or other similar effects were detected through the flow-rate profiles.
- The filter materials used for filtration and ultra filtration were chemically compatible with a wide range of solvents and with no adsorptive capacity for soluble organics in a way to interfere with segregation based on particle size.
- The first COD measurements were carried out for raw samples (not-settled, not filtered, but mixed), whereas the first COD measurements were done for the samples filtered through AP40 glass fiber filters to avoid any interference and impact due to absorption on/scattering from particulate matter.
- In this context, the particulate fraction was determined as part residual above AP40 glass fiber filter (41600nm), apparently comprising the settleable (4105 nm) and most of the supracolloidal (103–105 nm) substances in the sample.
- Leakage, composed after filtering through 1 kDa ultrafiltration membrane (<2nm), therefore including the greater part of the dissolved components (<1nm), which was consented as the soluble portion.
- Finally, the portion between the range of 2nm (1 kDa membrane) and 1600nm (AP40 glass fiber filter) was accepted as the colloidal range.(APHA, 1998)
- The schematic illustration of the sequential filtration and ultra filtration procedure presented in the Figure 3.2



(~nm):	2	3	5	8	13	220	450	1600
(kDa):	1	3	10	30	100			
	Ultrafiltration					Filtration		

Figure 3.2.: – Schematic presentation of the sequential filtration/ultrafiltration procedure: 1—Non-settled, non-filtered, but mixed original sample, 2—aliquot of filtrate from the previous step, subjected to COD measurements, 3—aliquot of filtrate from the previous step, subjected to subsequent filtration/ultrafiltration procedure, 4—gas line providing positive pressure. (Dulekgurgen, et al., 2006)

3.2.4. Toxicity Test Method

The algal toxicity test by using *Phaeodactylum tricornutum* (marine algae) as indicator species was applied for determination of toxicity of wastewaters. The principle of the test procedure was as explained in US EPA (1978) batch algal bioassays. The bioassays were applied to both raw and ozone-pretreated tannins. The experiments are carried out at room temperature (T=20 ±2^{0}C), at a pH of around 7, with the lightening by cool-white fluorescent lamps (3500-4000 lux) as described by Okay et al.. (2002) .*Phaeodactylum tricornutum* (marine algae) species were cultured in f/2 medium (Table 3.2)

Table 3.2.: Concentrations of f/2 Medium Solutions (Guillard.1972)

Sample No	Components	Concentration (g/l)
1	NaNO3	75
2	NaH2PO4.H2O	5
3	NaSiO3.5H2O	12.90
4	CuSO4.5H2O ZnSO4.7H2O CoCl2.6H2O MnCl2.4H2O	0.005 0.011 0.005 0.09
5	FeCl3.6H2O	0.909

Procedure of Toxicity Tests;

- The principle of batch toxicity tests is mainly based on the method of US EPA bottle test and was performed as previously described by Okay et al (2002).
- 13 gr NaCl was added to ozone-pretreated tannins to obtain the desired salinity concentration for survival of the marine algae
- Culture media was made of filtered clean seawater (0,45 µm-membrane Milipore) collected from the surface waters (20-22 ppt) of Marmara Sea (Istanbul Strait) known as relatively cleaner site and of modified f/2 nutrient medium (Guillard, 1972).
- Different dilutions with seawater were prepared for raw tannins (5%, 10%, 20%, 40%,80%). Diluted solutions of raw (500 mg/L in filtered sea water) and ozonated lignin sulphonic acid derivative are incubated together with the algal species in 250 mL glass
- The dilutions were prepared for pretreated tannins with addition of 13g NaCl/ l (2%, 5%, 10%, 20%, 40%)
- f/2 medium solutions were added as to be 1ml to 1L test solution to Erlenmeyer flasks
- The algal species of *Phaeodactylum tricornutum* were added to raw , pretreated tannins and also control samples as to be a starting concentration of 10000cells/ml
- The flasks were repositioned daily within the experimental space to minimize possible spatial differences in illumination and temperature on growth.

- Cells were counted daily with a Coulter Counter (Beckman Model Z 2) for 7 days for following of the algal growth.
- The EC values were evaluated from dose-response curves created for raw and pretreated tannins.

4. RESULTS AND DISCUSSION

The present study is based on a textile mill that performs wet processing especially on the dyeing processes. The three basic wet processed in stages of characteristic textile mills separately volumetric and pollution loads are viewed in Figure 4.1.

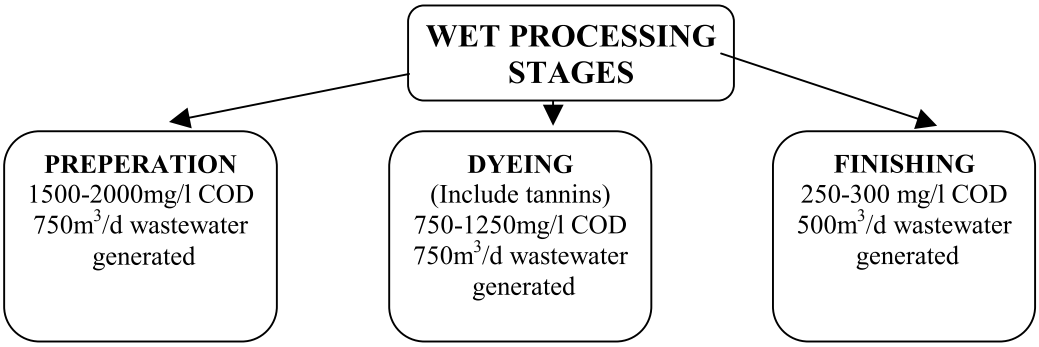


Figure 4.1.: Volume of Wastewater Generated and COD Load of Wet Processing Stages (Pisa Tekstil, 2004)

Textile mill generates 2000m^3/d wastewater; COD of dyeing and finishing wastewater is around 1100mg/l. In dyeing and rinsing processes approximately 750m^3/d wastewater is generated, contributing to almost 35% (900kg/d) of the total COD load discharging as textile effluent. Nearly 10% (270kg/d) of total COD load in wet processing stages is generated from finishing stage that has flow rate of around 500m^3/d.

The effluent from finishing stages causes serious treatment troubles since it contains containing a lot of toxic and recalcitrant finishing agents.

Dyes used by the textile industry are largely synthetic, typically derived from coal tar and petroleum-based intermediates. Dyes are sold as powders, granules, pastes, and liquid dispersions, with concentrations of active ingredients ranging typically from 20 to 80 percent (O'Neill et al., 2000a).

4.1. Conventional Characterization of Natural and Synthetic Tannin

In Table 4.1, the COD values together with the established for raw NT and ST. The experiments were performed on the samples which were prepared by mixing 1.25 g ST and 1.56 g NT to a liter of distilled water in order to simulate batch dyeing processes for each experiments

Table 4.1.: COD values for NT and ST

	NT	**ST**
COD mg/L	1100±100	460±20

It can easily be estimated from Table 4.1 that; COD value of synthetic tannin is the half of the one associated with NT.
Pretreatment with ozone for NT and ST are previously evaluated as Ozone Feeding Rate = 1000 mg/h; pH = 3.5; t = 40 min (Koyunluoğlu, 2005)

The COD values after pretreatment with ozone are given in Table 4.2.

Table 4.2.: COD of NT and ST after Treatment with Ozone:Experimental Conditions: Ozone Feed Rate = 1000 mg/h; pH = 3.5; t = 40 min.

Ozonation (pH = 3.5, 1000 mg/h)	**NT**	**ST**
COD mg/L	910 ± 30	280±25

In Table 4.2, the COD of NT and ST are given after ozone-pretreatment (40 minutes of ozonation at a feeding rate of 1000 mg/h and a pH of 3.5). With ozonation 83% of COD is treated for NT and 60 % of COD is treated for ST treated.

4.2. Inert COD Fractions

4.2.1. Raw Natural Tannin

Inert COD experiments run for 79 days in order to monitor threshold residual COD values in reactors. Table 4.3 tabulates the data on inert COD for raw NT.

Table 4.3.: Inert COD experiment results for raw NT

	Wastewater Reactor	Glucose Reactor
Initial COD (mg/l)	1097	1058
Final COD (mg/l)	100	75
Time(day)	79	79

At the beginning of the experiment as shown in Table 4.3 and Figure 4.2; the raw wastewater reactor was feed with NT having a COD of 1097mg/l. A COD reduction of 997 mg/l was observed for raw wastewater. On the other hand the glucose reactor had a COD of 1058 mg /l at the beginning. This value was decreased to a COD of 75 mg/l. Table 4.4 tabulates the COD fractionation of raw NT.

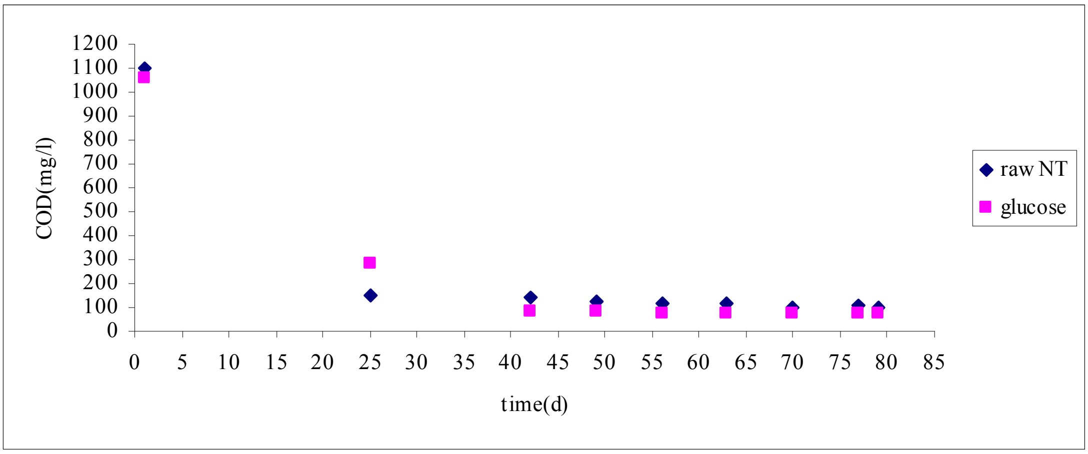


Figure 4.2. : The reduction of COD for raw NT in wastewater and glucose reactor

Table 4.4.: COD Fractions of Raw NT

Parameter	Raw NT (mg/l)
$S_{T0} = S_{S0} + S_I$	1097
$S_T = Y_{SP}\, S_{S0} + S_I$	100
S_{G0}	1058
S_{PG}	73
$Y_{SP} = S_{PG} / S_{G0}$	0.069
S_{S0}	1073
S_I	24
% S_I / S_{T0}	2

According to the experimental results demonstrated in Table 4.4; it can be observed that raw NT had an inert COD content of 24 mg/l COD, which involves the 2 % of NT solution. The S_T value of NT is 100mg/l COD which is the sum of S_I and S_P. In other words if NT is given to a well operated conventional biological treatment plant, the effluent of plant can not have a lower COD value than 100mg/l. Such a recalcitrant COD level will not cause serious problems in treatment plant.

4.2.2. Pretreated Natural Tannin

Inert COD experiments run for 54 days so as to monitor threshold residual COD values in reactors. Table 4.5 tabulates the data on inert COD for pretreated NT.

Table 4.5.: Inert COD experiment results for pretreated NT

	Wastewater Reactor	Glucose Reactor
Initial COD (mg/l)	923	972
Final COD (mg/l)	38	31
Time(day)	54	54

At the beginning of the experiment as shown in Table 4.5 and Figure 4.3, the pretreated wastewater reactor was feed with NT having a COD of 923 mg/l. A COD reduction of 885 mg/l was observed for raw wastewater. On the other hand the glucose reactor had a COD of 972 mg /l at the beginning. This value was decreased to a COD of 31 mg/l. Table 4.6 tabulates the COD fractionation of pretreated NT.

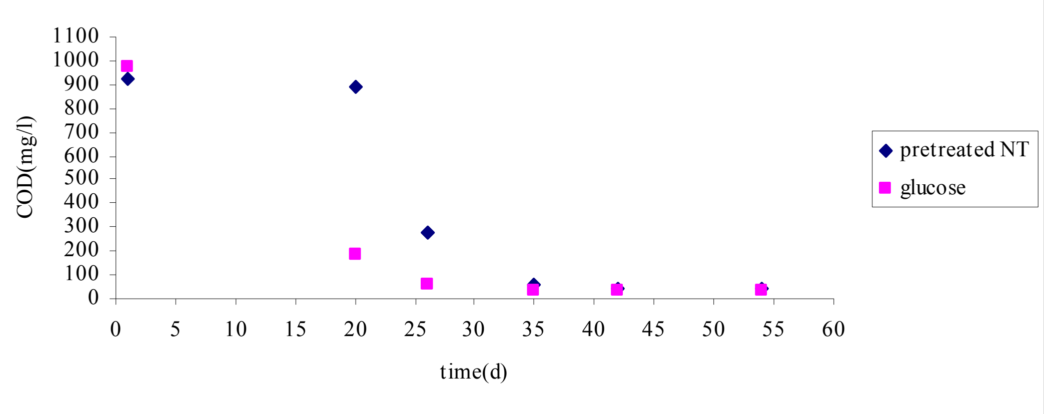


Figure 4.3.: The reduction of COD for pretreated NT in wastewater and glucose reactor

Table 4.6.: Inert COD Evaluation for Pretreated NT

Parameter	Pretreated NT (mg/l)
$S_{T0} = S_{S0} + S_I$	923
$S_T = Y_{SP}\ S_{S0} + S_I$	38
S_{G0}	972
S_{PG}	31
$Y_{SP} = S_{PG} / S_{G0}$	0.032
S_{S0}	914
S_I	9
% S_I / S_{T0}	1

According to the experimental results demonstrated in Table 4.5 and Figure 4.3, it can be observed that pretreated NT had an inert COD content of 31 mg/l COD, which involves the 3% of NT. As COD fractions are shown in Table 4.6, it can be observed that the S_T value of pretreated NT is 38 mg/l COD which constituted by the sum of S_I and S_P.

4.2.3. Raw Synthetic Tannin

Inert COD experiments run for 79 days in order to monitor threshold residual COD values in reactors. Table 4.7 tabulates the data on inert COD for raw ST.

Table 4.7.: Inert COD experiment results for raw ST

	Wastewater Reactor	Glucose Reactor
Initial COD (mg/l)	463	437
Final COD (mg/l)	188	60
Time(day)	79	79

As indicated in Table 4.7 and in Figure 4.4; at the beginning of the experiment the raw wastewater reactor was feed with ST having a COD of 463 mg/l. A COD reduction of 275 mg/l observed for raw wastewater. On the other hand the glucose reactor had a COD of 437 mg COD/l at the beginning. This value was decreased to a COD of 60 mg/l. Table 4.8 tabulates the COD fractionation of raw ST.

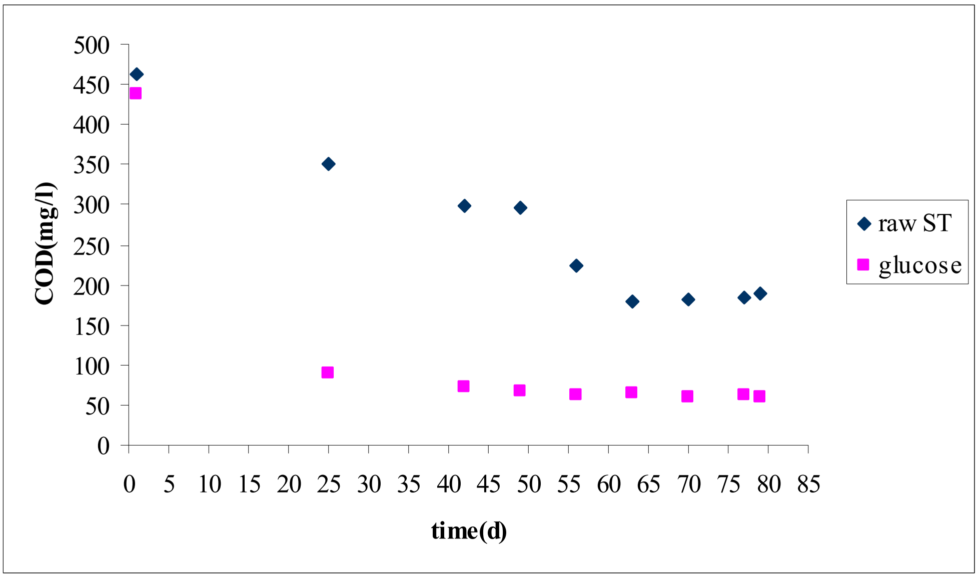


Figure 4.4.: The reduction of COD for raw ST in wastewater and glucose reactor

Table 4.8.: Inert COD Evaluation for Raw ST

Parameter	Raw ST (mg/l)
$S_{T0} = S_{S0} + S_I$	463
$S_T = Y_{SP}\, S_{S0} + S_I$	188
S_{G0}	437
S_{PG}	60
$Y_{SP} = S_{PG} / S_{G0}$	0.137
S_{S0}	328
S_I	135
% S_I / S_{T0}	29

According to the experimental results demonstrated in Table 4.8, it can be observed that raw ST had an inert COD content of 135 mg/l COD, which involves the 29 % of ST. The biocalcitrant COD concentration of 188 mg/l obtained at the outlet of the activated sludge system. Such a recalcitrant COD can be considered as a high level that requires a chemical oxidation prior to the biological treatment.

4.2.4. Pretreated Synthetic Tannin

The experiment run 54 days until pretreated ST and glucose reactors reach a threshold residual COD value. Table 4.9 tabulates the data on inert COD for pretreated ST.

Table 4.9.: Inert COD experiment results for pretreated ST

	Wastewater Reactor	Glucose Reactor
Initial COD (mg/l)	282	282
Final COD (mg/l)	93	64
Time(day)	54	54

As exhibited on the Table 4.9 and Figure 4.5; at the beginning of the experiment the wastewater reactor was feed with pretreated ST having a COD of 282 mg/l. A COD reduction of 189 mg/l was observed for pretreated wastewater. Whereas the glucose reactor had a COD of 282 mg COD/l at the beginning and this value was decreased to the 64 mg/l. Table 4.10 tabulates the COD fractionation of pretreated ST.

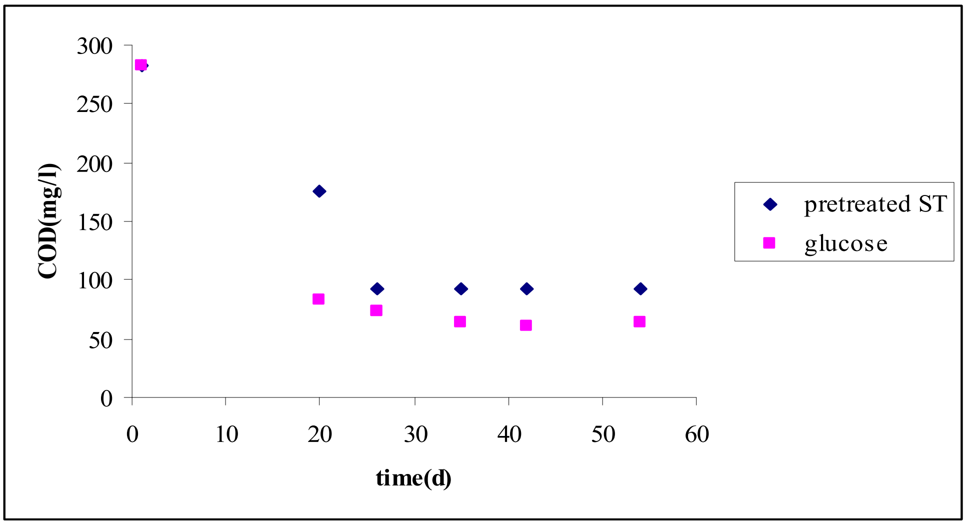


Figure 4.5.: The reduction of COD for pretreated ST in wastewater and glucose reactor

Table 4.10.: Inert COD Evaluation for Pretreated ST

Parameter	Pretreated ST (mg/l)
$S_{T0} = S_{S0} + S_I$	282
$S_T = Y_{SP} S_{S0} + S_I$	93
S_{G0}	282
S_{PG}	61
$Y_{SP} = S_{PG} / S_{G0}$	0.216
S_{S0}	241
S_I	41
% S_I / S_{T0}	14

According to the experimental results demonstrated in Table 4.10, it can be observed that pretreated ST had an inert COD content of 41 mg/l COD, which involves the 14 % of ST. The S_T value of raw ST is 93 mg/l, pretreatment is successful for decrease of S_T of wastewater.

4.2.5. Comparison of Inert COD Fractions

According to the inert COD fractions tabulated in Table 4.11., raw NT has a COD value that is two times higher than raw ST. However, if S_T values compared for both solutions; the result shows that the S_T value is 100mg/l COD for raw NT whereas, raw ST has an S_T of 188 mg/l. Raw NT can go through well operated conventional activated sludge without requiring any pretreatment, but raw ST must be pretreated prior to biological treatment.

Table 4.11.: Comparison of COD fractions for raw and pretreated NT&ST

	Y_{SP}	S_{T0} (Mg/l COD)	S_I (Mg/l COD)	S_T (Mg/l COD)	S_T/S_{T0} (%)
Raw NT	0.069	1097	24	100	<1
Raw ST	0.0137	463	135	188	40
Pretreated NT	0.032	923	9	38	4
Pretreated ST	0.216	282	41	93	14

If the effect of ozone pretreatment considered; the COD of NT decreases 15 %; whereas the S_I decreases 63 % after treatment. With ozone pretreatment 16% COD removal can be obtained for NT. As the raw NT sample has an S_I of 24mg/l corresponding to an S_T value of 100 mg/l, approximately the same removal percentage of 62 can be observed on both S_I and S_T with ozonation. The raw NT sample contains biologically recalcitrant COD content of 100mg/l that will appear in the effluent of biological treatment.

The ozone pretreatment is also successful for ST; the COD of ST was decreased 39 % ; whereas an S_I decrease of 70% was observed after treatment. Ozone pretreatment can be helpful for decreasing the effluent COD of biological processes because it successfully oxidizes S_I; and as a result the S_T will drop to a value of 93 mg/l COD.

4.3. Sequential Filtration/Ultra filtration Experiments

4.3.1. Raw and Pretreated NT

The COD distribution for raw and pretreated NT related to molecular weight cut-offs are presented in Table 4.12 and Table 4.13. In these Tables, the cumulative COD values of raw and pretreated NT below the selected filter sizes are illustrated. The experiments were last two days.

Table 4.12.: COD values of raw and pretreated NT related to molecular weight cut-off

Separation Technique	Particle Size (nm)	Cumulative COD (mg/l)	
		Raw NT	**Pretreated NT**
Total		1230	874
Filtration			
AP40 filter	1200-1600	1140	843
HV filter	450	811	811
GV filter	220	811	811
Ultra filtration			
100 kDa	13	631	811
30 kDa	8	631	811
10 kDa	5	598	811
3 kDa	3	598	811
1 kDa	2	561	811

Table 4.13.: Distribution of COD related to molecular weight cut-off for raw and pretreated NT

SAMPLE ↓	PARAMETER ↓	MOLECULAR WEIGHT CUT-OFF →	GF/D – TOTAL	0.45 µm– Ap40	0.22 µm–0.45 µm	100 kDa – 0.22 µm	30 kDa – 100 kDa	10 kDa – 30 kDa	3 kDa – 10 kDa	1 kDa – 3 kDa	< 1 kDa	TOTAL
*	COD	(mg.l^{-1})	90	329	0	182	0	33	0	37	561	1230
		(%)	7	27	0	15	0	3	0	3	45	100
**	COD	(mg.l^{-1})	31	32	0	0	0	0	0	0	811	874
		(%)	3	4	0	0	0	0	0	0	93	100
		Removal (mg.l^{-1})	59	297	0	0	0	0	0	0	0	356

* raw NT
** ozone pretreated NT Ozone Feed Rate = 1000 mg/h; pH = 3.5; t = 40 min.

Fingerprints of raw and treated NT based on differential COD values for selected particle size categories and percent COD distribution for raw and pretreated NT related to molecular weight cut-off are shown in Figure 4.6 and in Figure 4.7

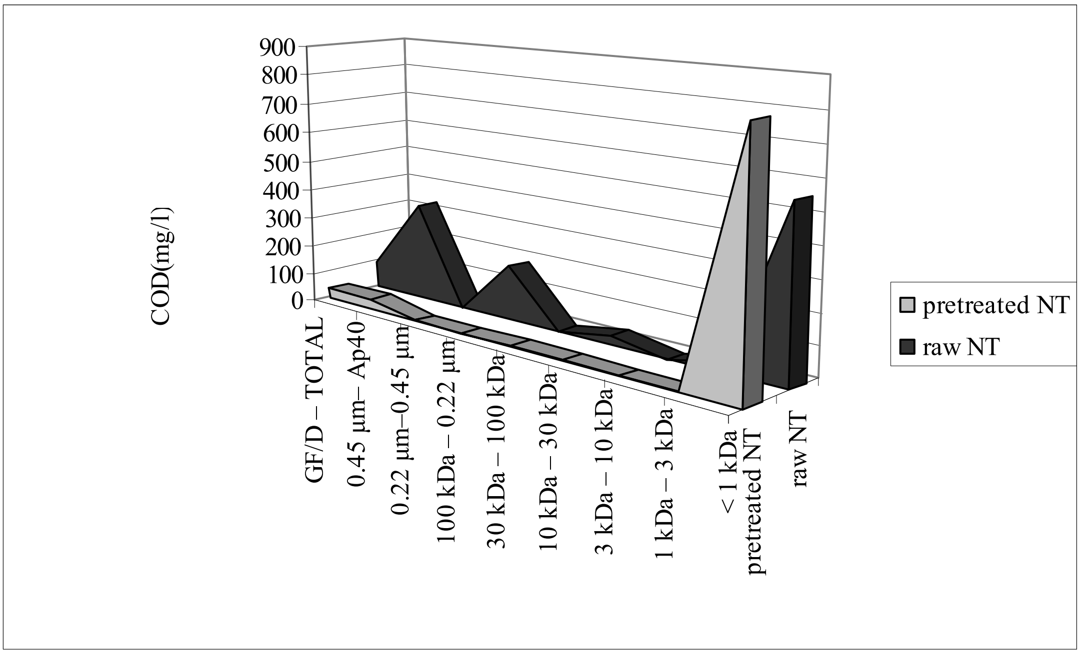


Figure 4.6.: Fingerprints of raw and treated NT based on differential COD values for selected particle size categories

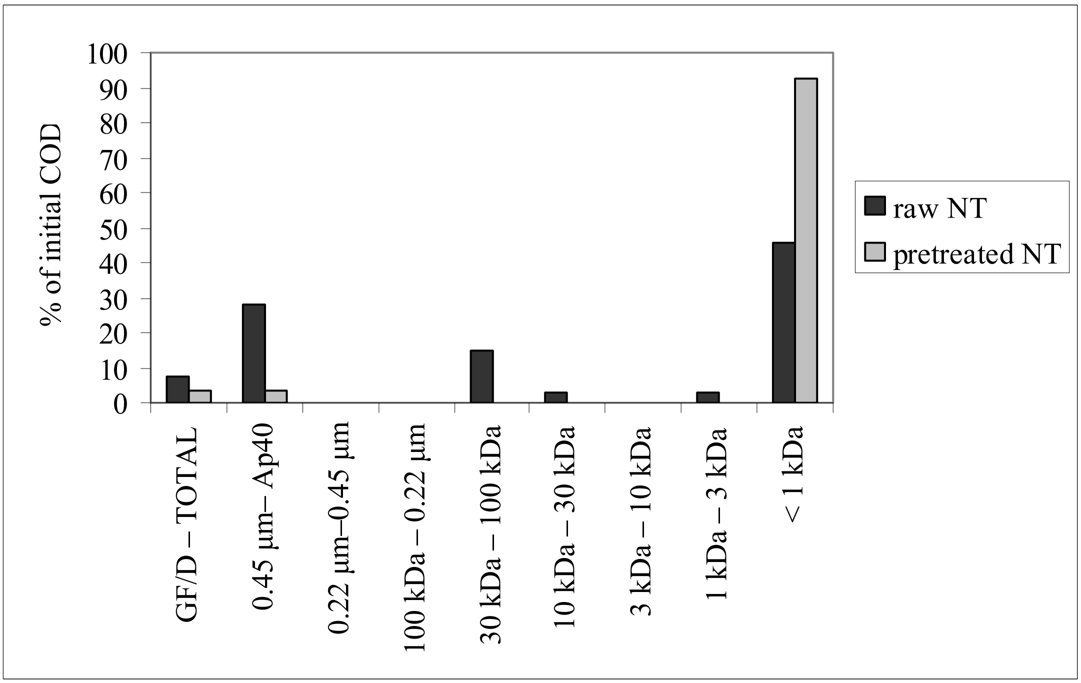


Figure 4.7.: Percent COD distribution of raw and pretreated NT related to molecular weight cut-off

According to the size distributions shown in Table 4.12., 45% COD of raw NT particulates are in dissolved range which have the size below 1kDa. The rest of particulates that make up 55% COD of raw NT can be grouped as settleable. However, 93% COD of pretreated NT particulates are in dissolved range.

As expected, big molecules were broken into small pieces by ozonation. According to differential COD values for selected particle sizes, it can be observed that dissolved particulates were increased after pretreatment.

4.3.2. Raw and Pretreated ST

The results of sequential filtration and ultra filtration experiments for ST are shown in Table 4.14. and Table 4.15. The experiments were last three days with the measurement of cumulative COD below different sized filtrates.

Table 4.14: Size Distribution of raw and pretreated ST

Separation Technique	**Particle Size (nm)**	**Cumulative COD (mg/l)**	
		Raw ST	**Pretreated ST**
Total		475	256
Filtration			
AP40 filter	1200-1600	457	248
HV filter	450	420	228
GV filter	220	420	228
Ultrafiltration			
100 kDa	13	420	228
30 kDa	8	420	228
10 kDa	5	420	228
3 kDa	3	420	228
1 kDa	2	420	228

Table 4.15.: Distribution of COD related to molecular weight cut-off for raw and pretreated ST

SAMPLE ↓	PARAMETER ↓	MOLECULAR WEIGHTCUT-OFF →	GF/D – TOTAL	0.45 µm– Ap40	0.22 µm–0.45 µm	100 kDa – 0.22 µm	30 kDa – 100 kDa	10 kDa – 30 kDa	3 kDa – 10 kDa	1 kDa – 3 kDa	< 1 kDa	TOTAL
*	COD	(mg.l^{-1})	18	37	0	0	0	0	0	0	420	475
		(%)	4	8	0	0	0	0	0	0	88	100
**	COD	(mg.l^{-1})	8	20	0	0	0	0	0	0	228	256
		(%)	3	8	0	0	0	0	0	0	89	100
		Removal (mg.l^{-1})	10	17	0	0	0	0	0	0	192	219

* raw ST

** ozone pretreated ST Ozone Feed Rate = 1000 mg/h; pH = 3.5; t = 40 min.

Fingerprints of raw and treated ST based on differential COD values for selected particle size categories and percent COD distribution for raw and pretreated ST related to molecular weight cut-off are shown in Figure 4.8 and in Figure 4.9

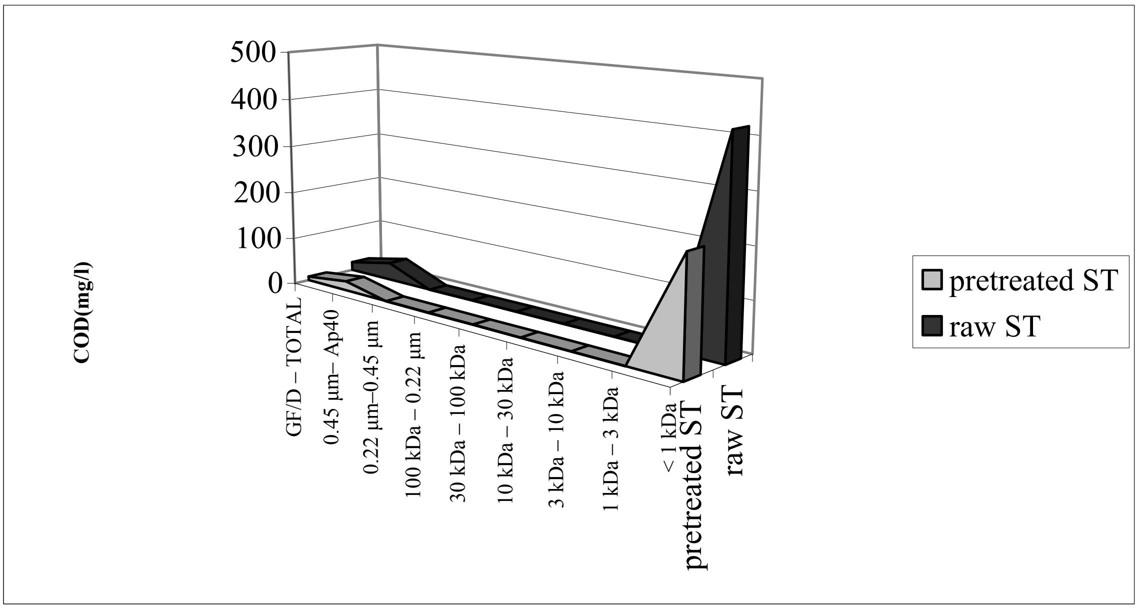


Figure 4.8.: Fingerprints of raw and treated ST based on differential COD values for selected particle size categories

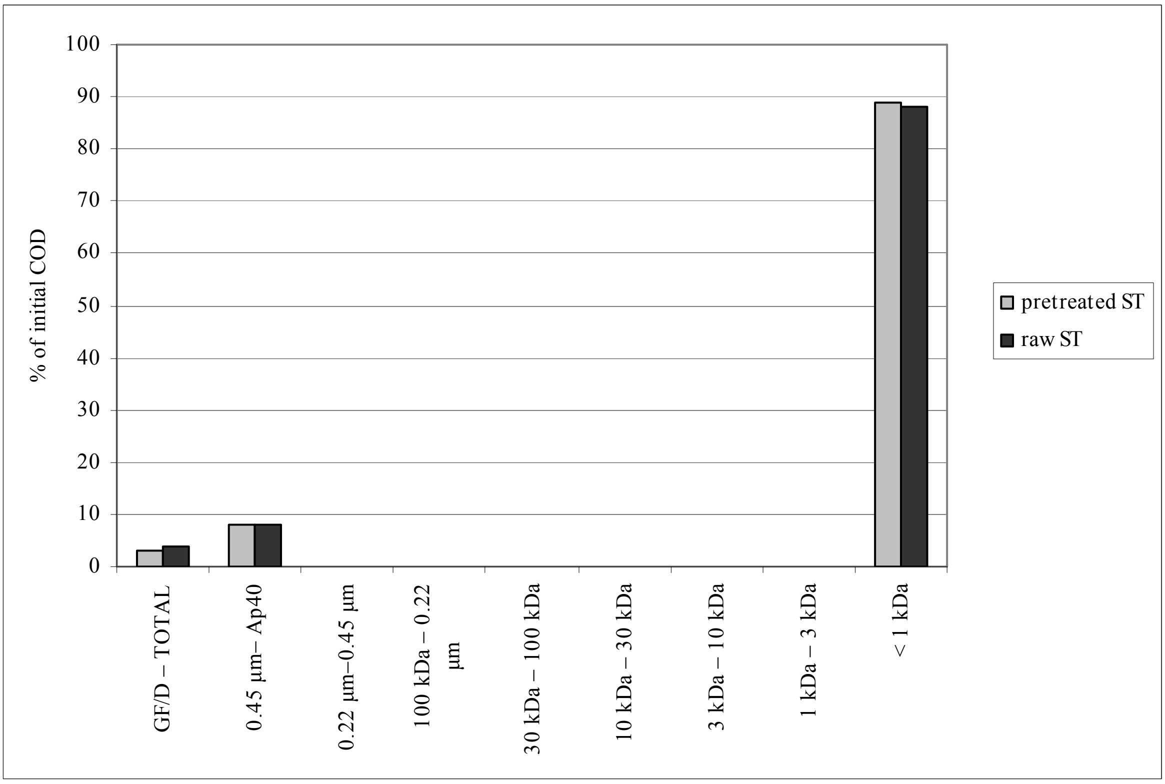


Figure 4.9.: Percent COD distribution of raw and pretreated ST related to molecular weight cut-off

According to the size distribution shown at Table 4.13 and 4.14; 88 % COD of raw ST particulates have particulate sizes below 1kDa that can be categorized as dissolved. The rest of COD that comprise 12% of raw ST are in settleable part. However, 89% COD of ST has in dissolved range after ozonation.

If this experiment results are evaluated with COD it can be interpreted that besides the common opinion that inert COD usually made up big molecules, the inert COD of ST has in dissolved range.

4.3. Toxicity Experiments

The percent inhibition values of *P. tricornutum* obtained for different dilutions of raw NT, raw ST, ozone-pretreated NT and ozone-pretreated ST detected after 7 days of exposure and results are summarized in Table 4.16.

Table 4.16. : Growth inhibition values (%)of *P. tricornutum* obtained for raw and ozone-pretreated formulations.

Sample	**Sample Dilution(%)**	**I *P. Tricornutum(%)***
Raw ST	5	98
	10	99.88
	20	99.55
	40	99.14
	80	99.59
Raw NT	5	99.17
	10	99.68
	20	99.74
	40	99.15
	80	96.98
Ozone-pretreated ST	2	22.08
	5	26.02
	10	37.95
	20	69.4
	40	84.17
Ozone-pretreated NT	2	10.05
	5	47.96
	10	53.54
	20	72.51
	40	89.23
	80	96.97

The experimental results show that, increasing the addition of tannin effluents cause an increase in the inhibition of algal activity. It can be observed from the obtained values that; raw NT and ST have much more toxic effect on *P. tricornutum* than that of ozone pretreated NT and ST. *P. tricornutum* species were inhibited as approximately as 100 % in all dilutions of ST formulation and percent inhibition of microalga is found as 97 % for NT formulation at a dilution of 80%.

According to the results, ozone-pretreated NT and ST samples were found much less toxic compared to the results obtained for raw NT and ST samples.

Dose-response curves of *P. tricornutum* exposed to ozone pretreated NT and ST samples are at different dilutions is shown in Figure 4.10.

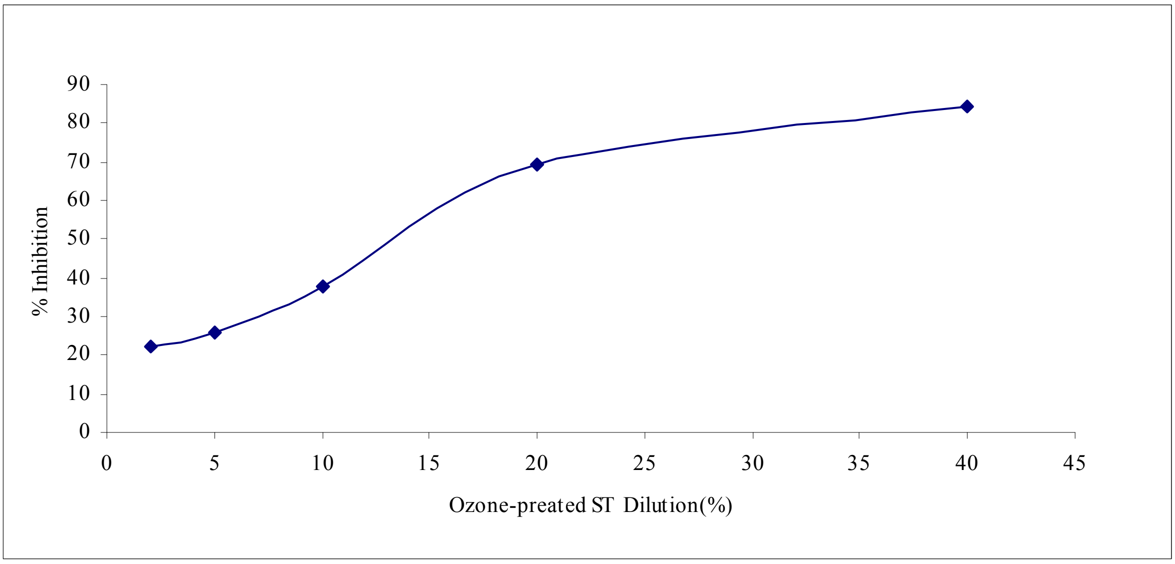


Figure 4.10.: Percent inhibition of P. tricornutum for ozone-pretreated ST samples at several dilutions.

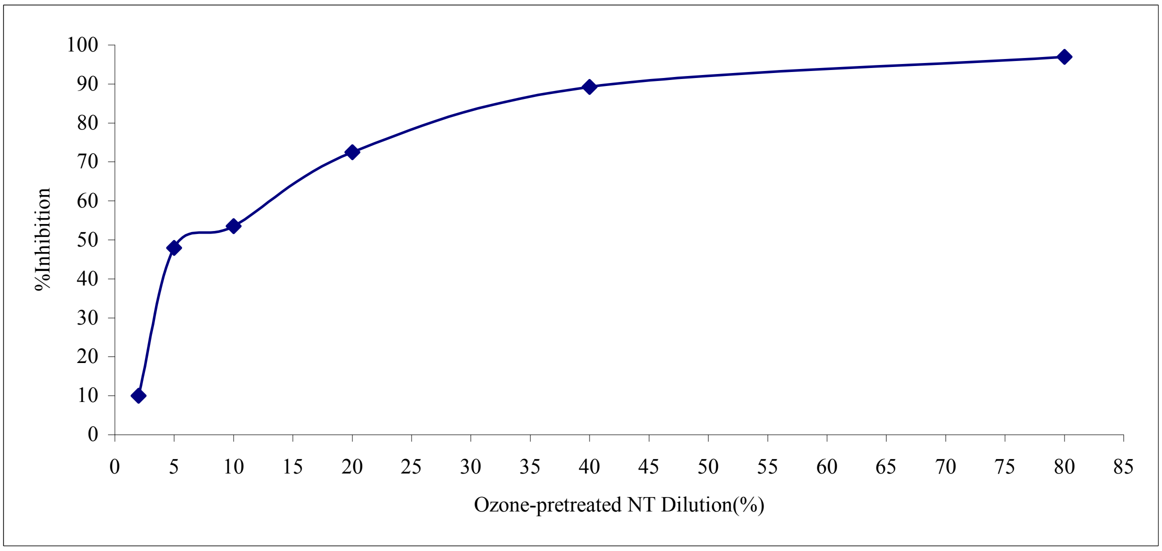


Figure 4.11.: Percent inhibition of P. tricornutum for ozone pretreated NT samples at several dilutions.

Figure 4.10 and Figure 4.11 shows the percent inhibition of *P. tricornutum* for ozone-pretreated NT and ozone pretreated ST samples at several dilutions.
Pretreatment: The ozone feed rate of 1000mg/h at a pH of 3.5 for 40 minutes is applied to NT and ST

EC values estimated from the dose-response curves after 7 days of exposure is given in Table 4.17.

Table 4.17.: EC values of raw and pre-treated Tannin Solutions After 7 days of Exposure to *P. tricornutum*.

TEST SUBSTANCE	**EC_{20}**	**EC_{50}**	**EC_{80}**
Syntan	<1	<1	<1
Natural Tannin	<1	<1	<1
Pretreated Syntan	2	13	34
Pretreated Natural Tannin	3	6	28

Raw tannins are extremely toxic so that EC values could not be found for those samples. From the Table it is clearly seen that ozone-pretreated samples are less toxic than that of raw samples tannins. For example, EC_{80} value of raw natural tannin and raw synthetic tannin is less than 1%. When ozone-pretreatment applied to raw tannins, EC_{80} value for pretreated natural tannin increased to 28; and, EC_{80} value for pretreated synthetic tannin increased to 34.

In conclusion pretreatment is necessary for decreasing the toxic characteristic of NT and ST. Furthermore, according to the experiment results carried out by using *P. tricornutum*, ozone pretreatment is an effective way to decrease the toxicity of tannins.

5. CONCLUSION AND RECOMMENDATION

In the present study; the effects of ozone pretreatment on the biological treatability and toxicity of raw natural and synthetic tannin solutions have been examined.

According to the characterization of raw NT and ST solutions following issues can be given:

- The raw NT solution was predominantly soluble with a COD of 1100 mg/l.
- The raw ST solution was also in soluble form having a COD of 460 mg/l.
- The inert COD test carried out with acclimated biomass has revealed out that raw NT solution did not contain a significant amount inert COD. An inert COD content of 24 mg/l, corresponding to only 2% of total COD was associated with raw NT. The residual COD content of NT solution indicating the lowest achievable COD after biotreatment was 100mg/l.
- The inert COD results were evaluated for ST solution, too. Although, the raw COD value was almost half of that of NT; ST had an inert COD content of 135 mg/l (29 % of total COD). The residual COD content of ST solution indicating the lowest achievable COD after biotreatment was 188 mg/l.
- A toxicity test was performed on both tannin solutions using marine algae (*Phaeodactylum tricornutum*) due to its common use in toxicity testing. Both of the raw tannins were determined to be extremely toxic after 7 days exposure. Raw NT and ST solutions have EC_{80} values that are less than 1%.

According to the results of a previous study (Koyunluoğlu, 2005) a pretreatment with ozone was applied to raw NT and ST solutions. As for both of the tannin formulations pretreatment with 1000 mg ozone /L-h at pH =3,5 at 40 minutes contact times gave the optimum outputs, the following experiments were performed on ozonated NT and ST samples:

- After ozonation process COD content was decreased from 1100mg/L to 910 mg/L for NT solution yielding a 20 % COD removal.
- After ozonation process the COD content of the ST sample was decreased from 460mg/L to 280 mg/L, indicating a 40 % COD removal.
- The inert COD content of NT solution was decreased from 24 mg/L to 9 mg/L after ozonation process. 63 % soluble inert COD removal was provided by this method. If the results were evaluated in terms of achievable residual effluent COD after biotreatment a decrease from 100mg/l to 38mg/l was observed.
- After ozonation process, the inert COD content of ST solution was decreased from 135 mg/L to 41 mg/L. By ozonation 70 % soluble inert COD removal was provided. The residual effluent COD achieved after biotreatment was decreased from 188mg/L to 93 mg/L with ozonation. After ozonation, the

residual COD of NT solution was decreased from 40% to 33% of total COD content.

- The studies were performed to determine the toxic effects of raw and pretreated samples on the marine microalga, *Phaeodactylum tricornutum* using laboratory-based toxicity bioassays. When ozone-pretreatment applied, EC80 value for pretreated natural tannin was increased to 28 %; and EC80 value for pretreated synthetic tannin was increased to 34 %.

With considering the experimental results the following discussions could be done;

- The toxicity test on *Phaeodactylum tricornutum* have revealed out that; both of the NT and ST solutions are too toxic towards *Phaeodactylum tricornutum* and after the ozonation process, NT and ST solutions became less toxic to *Phaeodactylum tricornutum*. In terms of obtained **EC50 and EC80 a**fter ozonation ST sample exerted less toxic effect on the *Phaeodactylum tricornutum* than NT sample.
- The sequential filtration/ ultra filtration experiments also carried out for raw and ozonated NT and ST samples. According to the differential COD values for selected particle sizes, it can be observed that the COD content of molecular weight cut-offs corresponding to dissolved nature were increased after ozonation.

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