Effect of Intermittent Operation of Lab scale Upflow Anaerobic Sludge Blanket (UASB) Reactor on Textile Wastewater Treatment

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“Fondly dedicate my dissertation to my 2 years old boy and my loving sister whose words and actions of support and affection inspired me to pursue my studies and complete my research”

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LIST OF ABBREVIATIONS

BOD	Biological Oxygen Demand
COD	Chemical Oxygen Demand
ECP	Extracellular Polymers
EC	Electrical Conductivity
ERP	Effluent Recirculation Pump
F/M	Food/Microorganisms ratio
F	Feeding Period
FTIR	Fourier Transform Infrared Spectroscopy
GLSS	Glass Liquid Solid Separator
HRT	Hydraulic Retention Time
IP	Influent Pump
MLSS	Mixed Liquid Suspended Solids
MLVSS	Mixed Liquid Volatile Suspended Solids
MBR	Membrane Bioreactor
NEQs	National Environmental Quality Standards
NF	Non-feeding Period
TN	Total Nitrogen
OLR	Organic Loading Rate
ORP	Oxidation Reduction Potential
PAAs	Primary Aromatic Amines
PAOs	Phosphorus Accumulating Organisms

RTWW	Real Textile Wastewater
SRT	Solids Retention Time
SLR	Solids Loading Rate
SMA	Specific Methanogenic Activity
SBR	Sequencing Batch Reactor
SMPs	Soluble Microbial Products
TSS	Total Suspended Solids
VSS	Volatile Suspended Solids
TOC	Total Organic Carbon
TAAs	Total Aromatic Amines
TKN	Total Kjeldahl Nitrogen
UASB	Upflow Anaerobic Sludge Blanket
UV-Vis	Ultra Violet-Visible
VSS	Volatile Suspended Solids
VFAs	Volatile Fatty Acids

ABSTRACT

Different intermittent phases were introduced during the continuous operation of UASB reactor treating simulated textile wastewater using single dye and optimized combination was operated for mixed dye solution. Initially the reactor was operated at organic loading rate (OLR) of 2 kg COD/m^3d OLR and HRT of 24 hrs using single dye feed to optimize the non-feeding period provided to reactor for maximum COD and color removal rates. 12 hrs feeding and 12 hrs non-feeding (12F/12NF) cycle gave suitable COD and color removal rates of 57.50 and 71.04% respectively. The similar cycle was operated using mixed dye feed with increase in dye concentration to 30 mg/L at same operating conditions. Maximum COD and color removal efficiency of 47.80 and 38.35% was obtained. The decreased removal rates for mixed dyes were due to possible presence of intermediate metabolites produced by chromogenic breakdown of dyes. The above condition was extended as consecutive 12 hrs feeding and 12 hrs non-feeding periods (12F/12NF/12F/12NF) at 48 hrs HRT and increased dyes concentration to 50 mg/L. Improved COD and color removal rates of 67.0 and 77.86% were achieved. Minimum VFA/alkalinity ratio of 0.49 was obtained at stable MLSS concentration of 7300 mg/L. Effluent TKN and orthophosphates concentration was maintained at 42.35 and 18 mg/L, respectively.

1. INTRODCUTION

1.1. Background

Multiple processes for synthetic or natural fabric production in various types of textile industries have the major concern related to use and release of hazardous chemicals especially those that generate the finished products and make their way to effluent streams. Major environmental risks associated with them include the residual dye color which has low fixation rate to fabric, low fastness properties and low attraction with fabric where their residuals generate hazardous and carcinogenic metabolites in effluent dye bath that becomes difficult to be treated. Much of scientific research is focused on the use of green technologies using end-of-pipe solutions for these intense problems for reducing chemical and energy usage.

Textile sector is the major economical backbone of Pakistan which is the 8th largest exporter of textiles in Asia. Having major influence on GDP of 9.5% and a source of income to 15 million people of Pakistan i.e. 30% of working force of the country, textile industry is currently facing many financial, economic, social and environmental challenges. In spite of the introduction of textile policy, its full implementation is unforeseen. It catches public attention towards the standpoint of pollution, despite earning a huge amount of foreign exchange. In environmental sector, one of the biggest challenges of textile industries is meeting the international environmental protocols. Almost every textile plant running in region emits toxic effluent into air as well as generates concerns for ground and surface water. The fate of toxic, carcinogenic chemicals released after passing through many complex bleaching, dyeing, printing and finishing processes varies from 100% retention on fabric to 100% discharge with effluent depending on characteristics of chemicals used and attraction between fabric and the dye used. Experts say that

textile wastewater carry considerable pollution load in terms of Chemical oxygen demand (COD), Biological oxygen demand (BOD), Total suspended solids (TSS), Total dissolved solids (TDS) and heavy metals. The values of these parameters are always found to be high as compared to National Environmental Quality standards (NEQs) set by the government.

Table 1: Textile processes producing hazardous textile wastewater

Processes Involved	Textile Effluent Characteristics
Singering, Desizing	High BOD and TSS, Neutral pH
Scouring	High BOD, TSS, alkalinity and temperature
Bleaching, Mercerizing	High BOD, TSS and alkalinity
Heat-settling	Low BOD and TSS, alkaline wastewater
Dyeing, Printing & Finishing	Wasted dyes, High BOD, COD and TSS, neutral to alkaline wastewater

Source: Carmen & Daniela (2010)

Untreated effluent from dyed textile production is highly colored and thus, objectionable and offensive, if discharged into the open streams without being treated properly. Even though the dye concentration is well below 1 ppm i.e., its concentration lower than many other hazardous chemicals, dye is highly visible through naked eye and it becomes the top parameter to be detected in such wastewater. Many physical, chemical and biological conventional techniques are reported to be competently involved in removal, alteration, or isolation of these pollutants. However, these technologies are reported to be expensive, uneconomical and not feasible to get

installed at large scale in developing countries like Pakistan, who is facing serious financial and economic constraints (Charmy, 2006).

Table 2: Characterization of polluting effect of chemicals used in textile industry

Chemicals used in Textile Industry	Pollutants Features	Pollution Class
Alkali, mineral acids, salts, oxidants	Inorganic pollutants	1
Sizing agents, oils, waxes, organic acids, biodegradable surfactants, reducing agents	Easy biodegradable with moderate to high BOD	2
Colorants, whitening agents, impurities of polymeric nature, synthetic polymeric resins, silicones	Tough biodegradability	3
Polyvinyl alcohols, mineral acids, anionic or non-ionic emollients	Tough biodegradability with moderate BOD	4
Formaldehydes, colored compounds, accelerators, retarders, cationic emollients, complexants, salts of heavy metals	Cannot be removed by conventional biological methods, low BOD	5

Source: Carmen & Daniela (2010)

Dyes manufacturing companies, using progressive advancement in technology, emphasize on the dyes production, having complex chemical structures and diverse properties, to obtain the high fixation rates by dyeing or printing fabrics or other fibers with minimal usage of dyes. These dyes are designed to avoid the natural biodegradation by sunlight, water, any chemical or microbial action to attain long lasting bright colors on fabrics and maintain enhanced stability

(Wijetunga et al., 2010). In general, textile fibers adhere to dyes by Van-Dar Waals forces, hydrogen bonds or hydrophobic interactions (physical adsorption). But the strongest dye-fiber affinity is due to covalent bond with additional electrostatic interactions (chemisorption). Different types of cellulosic fibers combined with synthetic filaments exhibit different dyeing characteristics. Although the premium dye merchandizers claim to attain more than 80% of dye fixation rates on all fibers from light, medium to darker shades. The amount of dye usage increases exponentially for darker shades with decreased chance of fixation rates. Also, most of dyes, used in hydrolyzed form to increase their solubility, lose their adsorption properties to some fibers (Shaw et al., 2002). Poor exhaustion properties of various dyes used in dyeing processes cause almost 40% of initial dyes in unfixed hydrolyzed state (Lee & Pavlostathis, 2004; Manu & Chaudhari, 2002) while 10 – 20% of remaining dye is also lost in effluent after completion of dyeing process (Poonam et al., 1996). In alkaline conditions (i.e. pH 9-12), at high temperatures (30-70ºC), and salt concentration (40-100 g/L), reactive dyes form a reactive vinyl sulfone ($-SO_3$ $-CH=CH_2$) group, which forms a bond with the fibers. However, the vinyl sulfone group undergoes hydrolysis (i.e. a spontaneous reaction that occurs in the presence of water), and since the products do not have any strong affinity with the fibers, they do not form a covalent bond (Carmen & Daniela, 2010). Therefore, a high amount of dye residuals are discharged in the wastewater streams. The fixation efficiency varies with the class of azo dye used, which is around 98% for basic dyes and 50% for reactive dye (Carmen & Daniela, 2010). This leads to sever contamination of surface and ground waters in the vicinity of dyeing industries. The problem of high colored effluent has become recognized particularly with the dyeing of cellulose fibers (cotton which is 50% of the total consumed fibers in the textile industry worldwide) (Carmen & Daniela, 2010). Thus, it was reported that the degree of fixation

rate is never complete in textile dyes baths (Verma et al., 2015) resulting in effluent, containing various chemically complex dyes, making it one of the severely polluted wastewater, needed to be treated.

Table 3: Fixation rate of different dye classes

Dye Class	Fiber Type	Fixation degree (%)	Loss in effluent (%)
Acid	Polyamide	80-95	5 - 20
Basic	Acrylic	95-100	0-5
Direct	Cellulose	70-95	5-30
Disperse	Polyester	90-100	0-10
Metal complex	Wool	90-98	2-10
Reactive	Cellulose	50-90	10-50
Sulphur	Cellulose	60-90	10-40
Dye -stuff	Cellulose	80-95	5-20

Source: Carmen & Daniela (2010)

It was reported that 90% reduction in residual dye concentration in effluent dye bath has not been achieved either by reducing the usage of dye or eluding the discharge of textile effluent. Amongst different physical and chemical decolorization techniques, biological treatment has been found to be economically attractive (Firmino et al., 2010).

Diluted textile wastewater (TWW) contains various categories of dyes either based on their chemical structure (Azo, Anthraquinone, Azine, Xanthene, Nitro, Phthalocyanine, etc.) or application methods used (Acid, Basic, Direct, Reactive, etc.) (Wijetunga et al., 2008). They can be effectively treated by aerobic biological treatment technologies i.e. activated sludge process

where these dyes can get adsorbed on weak negatively charged aerobic sludge particles (Firmino et al., 2011) or converted into flocs due to their insoluble nature (disperse and vat dyes), their large molecular weight (direct dyes) or their cationic nature (basic dyes). However, dyes having 60 – 70% of market share (Couras et al., 2015; Farajzadehha et al., 2012) used for printing and dyeing, are hydrophilic, reactive and electron deficient in nature (anionic acid dyes) which do not get adsorbed on aerobic sludge easily (Firmino et al., 2011). They are less susceptible to oxidative catabolism (Manu & Chaudhari, 2002) and get hydrolyze easily having relatively less affinity for fiber (dos Santos et al., 2007) resulting in unfixed reactive dyes (Couras et al., 2015; G. & L., 2014).

The anaerobic reduction of azo dyes to simpler compounds has been well researched. These studies, have all reported the ability of anaerobic microbial sludges to efficiently reduce azo dyes to their intermediate metabolic structures, thus destroying the apparent visible color. Many of these intermediate products are aromatic amines with constituent side groups. Reduction of dye compounds to their intermediates solves the problem of aesthetic pollution, but more deleterious problems are created. Most azo dyes are non-toxic, but a higher percentage of their intermediates have been recognized as carcinogens. Because of the toxic potential of many aromatic amines, further degradation of the dye compound is necessary if toxicity is to be completely eliminated (Wallace et al., 2001).

However, some studies reported that aromatic reactive dyes having substituents containing nitro and sulphonic groups are quite recalcitrant to aerobic bacterial degradation (dos Santos et al., 2007). Few exceptional studies supported that biological degradation of aromatic amines is probable under coupled aerobic and anaerobic conditions (Couras et al., 2015; dos Santos et al., 2007; Işik & Sponza, 2005a; Muda et al., 2010). It has been reported that colorless aromatic

amines having hydroxyl and carboxyl groups can be fully mineralized under anaerobic methanogenic conditions (Somasiri et al., 2006). Color removal has been found to increase as the HRT of anaerobic phase increases in coupled anaerobic /aerobic treatment options (Muda et al., 2011). So, under strict anaerobic conditions, decolorization of dyes can be achieved and well documented (Manu & Chaudhari, 2002).

1.2. Objectives

Direct application of anaerobic processes in textile wastewaters treatment has been limited (Işik & Sponza, 2008). However, decolorization of dyes under strict anaerobic conditions are well documented (Şen & Demirer, 2003). In this research, Upflow Anaerobic Sludge Blanket reactor was used as primary and sole treatment option to treat the synthetic textile wastewater anaerobically. To achieve optimum results, continuous and intermittent mode of operation was observed for effective decolorization by varying the feeding and non-feeding period to anaerobic consortium. The reactor working efficiency was observed for mixed dye synthetic wastewater as well. The major objectives of research were:

1) Develop UASB reactor treating textile wastewater using continuous and intermittent operation.
2) Evaluate UASB reactor performance during various continuous (feeding period) and intermittent (non-feeding) operational conditions
3) Optimize intermittent (non-feeding period) operation of UASB reactor for maximum COD and color removal rates.

2. LITERATURE REVIEW

Complex wastewater consisting of numerous combinations of non-biodegradable dyes and a wide range of hazardous components has been a subject to diverse studies and numerous technologies but not even one explanation is capable to describe about a comprehensive treatment. Despite of awareness of this problem, many manufacturers are still unable to create the dyes which have increase dye-fiber bonding and lower losses with less requirement of additional chemical requirements for broad diversity of textiles (Wallace et al., 2001). Since it is unavoidable to detect these compounds in textile effluents, it is essential to have basic understanding of dyes used in the industry to recognize the pathway of breaking down the main structure of dyes into safer by-products. Thus, safety is ensured by involving appropriate technologies for human health and environment.

2.1. Dyes Classification

The vast spectrum of colorful dyes enveloping our surroundings is an amalgam of simple natural and complex compounds. This amalgamation of organic and inorganic compounds are released into wastage as pollutants and have been segmented into categories on the basis of chemical structure, reactivity to particular fabrics and the respective application method by dye manufacturing industry (Carmen & Daniela, 2010).

In order to grasp in depth knowledge of dyes, its general classification is divided into natural and synthetic/manmade dyes.

2.1.1. Natural Dyes

Ever since the beginning of 2600 BC up till almost 1856, dying sector mainly used natural dyes. Extracted out from vegetable and animal bulk was the eminent source of natural dyes. This extraction procedure was conveyed back in China. In 15^{th} Century BC, sea snails gave Tyrian and Indigo plant was the source of indigo color back in the 3000 BC. Enveloped and colored; Egyptian mummies' clothes used dyes from madder plants and Incas fine textures in South America (Carmen & Daniela, 2010).

2.1.2. Synthetic Dyes

W.H Perkin was the founder of manmade dye fabrication in 1856 and discovered mauve dye (aniline) giving a bright fuchsia color. Manufactured by P. Gries, azo dyes came forward as a result of diazotization reaction. Being aromatic compounds in nature, synthetic dyes possess aromatic rings in structure pertaining de-located electrons and diverse range of functional groups connected with it. Based on naphthalene, benzene or antracene rings, chromophore consists of aromatic structure bond with double conjugated links and de-located electrons in chromophores. The conformation of chromophore is categorized by azo group (-N=N-), ethylene group (=C=C=), methane group (-CH=), carbonyl group (=C=O), carbon-nitrogen (=C=NH; -CH=N-), carbon-sulphur (=C=S; ≡CS-S-C≡), nitro ($-NO_2$; -NO-OH), nitrozo (-N=O; =N-OH) or chinoid groups. The binding capacity of dye to the textile product is due to ionizable electrons or auxochrome which make up common auxochromes $–NH_2$ (amino), -COOH (carboxyl), $-SO_3H$ (sulphonate) and -OH (hydroxyl) (Carmen & Daniela, 2010)

Further categorization of textile dyes is sorted into two different ways:

1) According to application characteristics (i.e. acid, basic, direct, disperse, mordant, reactive, sulphur dye, pigment, vat, azo insoluble)
2) According to structure (i.e. nitro, azo, carotenoid, diphenylmethane, xanthene, acridine, quinolone, indamine, sulphur, amino- and hydroxy ketone, anthraquinone, indigoid, Phthalocyanine, inorganic pigment, etc.)

On the basis of general structure, textile dyes are also classified as anionic, nonionic or cationic (Carmen & Daniela, 2010). The dyes which give perplexing combination are brightly colored, water soluble reactive and acid dyes. The key anionic dyes are direct, acid or reactive dyes and are tough to remove from predictable biological treatment systems. Disperse dyes are core dyes which cannot ionize into aqueous solutions. Azo dyes which consist of azo, basic, anthraquinone disperse and reactive dyes etc. are the core cation dyes. Dyes are carcinogenic in nature containing aromatic or benzidine rings which are irrepressible to degradation due to their fused aromatic ring structure. Only a few disperse dyes can bio-accumulate where azo and nitro compounds are reduced in sediments while all other dye accumulating substrates to toxic amines e.g.

$$R1\text{-}N{=}N\text{-}R2 + 4H^{+} + 4e^{-} \longrightarrow R1\text{-}NH_2 + R2\text{-}NH_2$$

2.2. Azo Dyes

Comprising of 65-75% of total textile dyes, the most generally used textile dyes can be categorized in two classes, azo and anthraquinone. The azo dyes are further categorized by reactive groups: covalently bonded with HO-, HN-, or HS- functional groups bonded with fibers. Yellow, orange and red shades are the chief azo dyes color ranges. Anthraquinone happens to be

the second most used class of textile dyes containing a diverse and wide range of colors in visible spectrum and a particular color range for violet, blue and green (Carmen & Daniela, 2010).

Despite varied functional groups attached to azo dyes; they contain at least one nitrogen-nitrogen (N=N) double bond in their structure. Mono-azo dyes have only one N=N double bond, while di-azo and tri-azo dyes contain two and three N=N double bonds, respectively. Despite being bonded to aromatic heterocycles or aliphatic groups, the azo groups are mostly attached to benzene and naphthalene rings. With diverse spectrum of shades and respective intensities, the side functional groups ensure enforcement of dye color on every desired fabric.

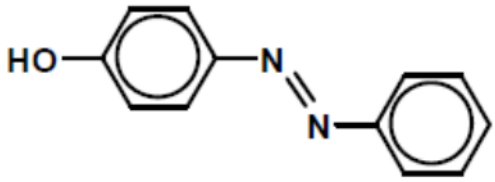


Figure 1: Structural formula for azo dye (derivative of diaryl azo compounds)

In a dye molecule, auxochromes can also be known as nucleophiles while the aromatic ring in the dye structure is chromophore. Together, they make the dye called as chromogen. The color of dye visible is due to absorption and reflection of visible and UV irradiation. Mostly azo dyes undergo diazotization of primary aromatic amines, chemically bonded with single or multiple nucleophiles. Mostly amino and hydroxyl groups are bonded with the aromatic amines. So, huge variation of structurally different azo dyes can be found in textile manufacturing industry due to multiple structural combinations (Wallace et al., 2001).

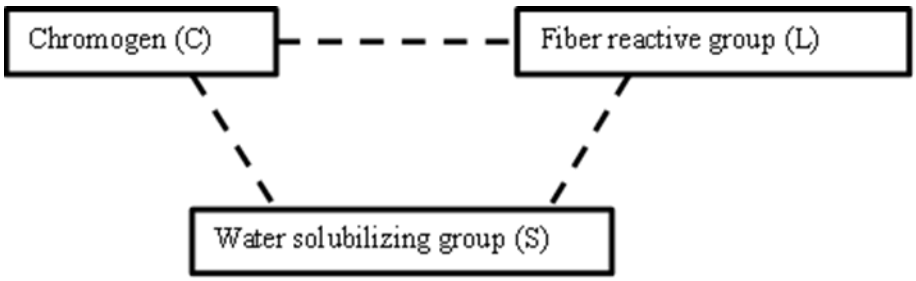
The molecular structure of chromogen group consists of 80-95% of azo dyes. The influential reactivity against covalently bonded fiber bonds pertain one or more functional groups in the

dye's structure and composition. They give fiber colors due to this bonding i.e. a carbon or phosphorous atom of the dye molecule will bond to hydroxyl groups in cellulose, amino, thiol, and hydroxyl groups in wool, or amino groups in polyamides. Due to this, dye shows high fastness and stability properties to substrates. Fiber reactive azo dyes are schematically shown as follows:

Where S= water solubilizing group, C= chromogen, B= Bringing group, R= Reactive group,

L= Fiber reactive or leaving group (Wallace et al., 2001).

The bridging group (B) combines the chromogen (C) with the reactive group (R) of the dye molecule. The bridging group (B) must be stable, water soluble, and highly flexible. For this, amino and alkyl amino groups are generally used. The reactive group functions to bond the dye molecule to a substrate via nucleophilic substitution or addition. Mono-, di-, and trichlorotriazinyl are all examples of reactive functional groups (Wallace et al., 2001).



2.2.1. Reactive Dyes

An intensely increased amount of dyes on wool and nylon are brought into use for dying cellulosic fibers which makes it 1/3rd of dyes used in textile industry. For cellulose, typical

functional groups are pyrimidine groups, triazine groups or sulphones where reaction occurs with hydroxyl groups of the cellulose by either nucleophilic substitution or addition.

Using Na_2CO_3 or NaOH, a pH of 9.5-11 is maintained when dying cotton fibers. Water becomes inert when upto 40 percent of dyestuff has been hydrolyzed. Thus, difficult to isolate (water soluble), reacted (inert) dye in waste water steams is found. For elevated dye bath exhaustion, common salt (NaCl) is brought into use. The electrolyte subdues negative charge build-up on the fabric caused by the use of anionic dyes and, hence, encourages increased dye uptake. The mandatory loadings differ with dyestuff especially when 100 g/L of dye liquor concentration is used. With its decline in usage since the early 1990s, urea was generally used as a second solvent. However, achievement of high fixation rates is not a requisite of urea for modern dyes.

A more predictive issue with reactive dyes is poor dye fixation and its minimum fastness property to the fabric. Due to excellence of shade reproducibility, its use is brought into continual use. To lighten the environmental impact as a result of dying, research is done. Water consumption is minimized and the need of electrolyte requirement is relieved by using either cationic dyes or a cationic polymer based pre-treatment. After dyeing, the fabric is repeatedly washed with surfactants to remove excess or unfixed dyestuff.

2.2.2. Toxicity Consideration

It is pivotal to consider the discharge of toxicity after degradation of dyes into their intermediate structures before choosing an active technology for complicated dyed effluent treatment. Human health and aqueous environment has been of concern to multiple researchers, reporting their apprehension on the terminal effects of dye and dye metabolites. Dye manufacturing industries found increased rates of bladder cancer in their workers in 1895. Substituted benzene and

naphthalene functional rings have been documented as carcinogenic agents because azo dyes predominantly found aromatic amines in their structures. A extensively huge percentage of degraded intermediate dye products are fatal for human health even when majority dyes are non-toxic in nature (Wallace et al., 2001). Basically 10% of diverse commercial textile samples were found to be mutagenic in nature as per an indicated research (Wallace et al., 2001). Combined from varied textile finishing plants, an inquiry on 45 effluents was conducted to disclose 27% of mutagenic Ames test samples. The disposal of gigantic variety of commercial dyes available makes it impossible to inspect all dyes thoroughly. Impurities found in commercial dyes manufactured and additives used also needed to be investigated further. For adding fiber-reactive dyes bonding onto substrates large amounts of salt are used. Leading to acute and chronic toxicity issues, these compounds are liable to cause intense electrolyte and conductivity concentrations in the dyed effluent.

Comprehensive information of dye structure and its respective degraded intermediate metabolites is unmanageable. Wallace et al., (2001) managed to gather a three-part list of mechanisms which were accountable for mutagenic activation of azo dye compounds. They are as follows:

1) Fabricating aromatic amines, azo dyes could be toxic only after reduction and cleavage of the azo linkage.
2) Azo dyes containing structures of free aromatic amine groups can be oxidized without azo reduction causing cancer.
3) Azo dye toxicity may also ensue by direct oxidation of the azo linkage creating extremely reactive, electrophilic, diazonium salts.

2.3. Comparison on Treatment Technologies

There are many researches that were recognized with diverse accent on biological, physical or chemical solutions for the treatment of composite dyed effluents. There is clear sign of achievement of these treatment technologies for having possibility for complete treatment of dye bath wastes. However, complete remediation was controlled due to couple of reasons involved. Chemical treatment technologies were applied at narrow level as they were expensive, while physical treatments cause massive amount of sludge production. Among them, biological treatment was proved to be effective in treatment of dyed effluents with low operating cost and lower sludge production. However, various combination of physical, chemical and biological techniques are proven to be very effective (Wallace et al., 2001).

Different treatment combinations of wastewater treatment were examined for effective action of textile wastewater to attain complete removal of color and organic compounds. Fenton's regent (H_2O_2 and Fe^{+2}), H_2O_2 and ozonation, are some advanced oxidation processes described for complete effective color removal but their working costs and sludge production is high. Other chemical technologies like coagulation and flocculation using lime, alum, polyelectrolyte and ferrous salts also produce large sludge, making it difficult for handling and disposal problems. The adsorption technologies used for dyes using activated carbon and other adsorbents are costly and requires pretreatment, making it extravagant solution to use. Reverse osmosis, requires high pressure and increasing energy requirements though it is effective in textile treatment. Membrane filtration methods i.e. nano-filtration and ultra-filtration techniques have high working costs and high sludge loads, making it uneconomical solution. The photochemical oxidation of dyes using UV with oxidation agents like catalysis, H_2O_2 are also costly and forms harmful by-products (Manu & Chaudhari, 2002). Electrochemical oxidation is a feasible

technique because it does not produce toxic by-products and not have higher color removal rates. But, high energy costs limit its application for the textile waste water treatment and makes it unfeasible to use (Somasiri et al., 2008). It is a slow and complicated process, difficult to get fully understood and applied (Manu & Chaudhari, 2002).

Microbial decolorization and degradation of organics is another cost-competitive technology to chemical and physical degradation process of dyed effluent (Senthilkumar et al., 2011). Microbial biodegradation is an economic and efficient treatment method. Pure cultures of fungi, bacteria or algae are stated to be efficient in complete decolorization of specific dyes but it is not applied at large scale. In the same way, conventional aerobic processes alone are not suitable for textile treatment as they cause no or poor decolorization especially decolorization of azo dyes (Somasiri et al., 2008). Since azo and reactive dyes are electron deficient in nature, this property makes them less susceptible to oxidative catabolism. They are also hydrophilic in nature and hence, pass through the conventional aerobic process untreated (Manu & Chaudhari, 2002; Ong et al., 2005a). Combination of anaerobic and aerobic processes has been shown as effective methods in removing color and organic loads in textile wastewater (Somasiri et al., 2008).

Due to the electron removal nature of the azo dyes, they are unsusceptible for aerobic oxidative catabolism and are not decolorized in aerobic processes alone. However, in the presence of co-substrate like glucose, and under anaerobic conditions, decolorization of azo dyes is greatly achieved with cleavage of the azo bond, thus making the azo dyes colorless, with or without formation of corresponding aromatic amines (Iik, 2004; Işik & Sponza, 2005b).

2.4 Granulation of Anaerobic Sludge

A physicochemical accumulation of vigorously growing biomass to form a large density and highly settable sludge is granulation process. Effective performance of UASB reactor is highly based upon the rate and extent of granulation. It is the indication of successful startup of anaerobic treatment process (Ghangrekar et al., 2005). Sludge granules are thick, multispecies microbial communities and it is impossible to completely degrade complex organic substrate with individual granular species.

2.4.1 Merits of Granular Sludge

The advantages of granular sludge are: (Ghangrekar et al., 2005; Latif et al., 2011; Nnaji, 2014)

1) Reduced washout possibility
2) Accelerated UASB reactor startup phase
3) High settleability ranges from 2-90 m/hr.
4) High methanogenic growth rate
5) High specific methanogenic activity
6) Low operating cost and reactor volume
7) Low inhibitors sensitivity
8) High oxygen tolerance
9) High SRT providing maximum microorganisms to space ratio
10) High OLR application

It is a main feature of granular sludge to accumulate microbes into symbiotic multilayer structure called granules and to control high vigorously growing biomass with good settling ability in

UASB reactor. Achievement of high OLR attaining sustainable operation is possible due to formation of high stable granules (Latif et al., 2011).

2.4.2 Granular vs. Flocculent Sludge

Flocculent sludge may form when the reactor is subjected with dilute influent or reactor having low upflow velocities, because this type of sludge has lower mass transfer resistance as compared to granular sludge. As a result of this, substrate is readily available to biomass. However, granular sludge settle effectively due to their large sizes than flocculent sludge which remain suspended in reactor, leading to abrupt washout. The microbial complexity in granules makes it less resistance to chemical pollutants inhibition as compared to flocculent sludge (Buzzini & Pires, 2007). Since flocculent sludge has lower mass transfer resistance due to dilute incoming influent, this explains the higher SVI values for this sludge subjected to lower loads (Couras et al., 2015).

2.4.3 Granulation Process

This process can be explained as transport, adsorption, adhesion and multiplication. However, the first step of granulation is acclimatization of microbes to substrate, followed by microbial multiplication in form of granules. A typical granules is identified by Methanosaeta Spp. mainly, inorganic deposits, protein, polysaccharides and nucleic acid having chemical formula of $C_7H_{12}O_6.6N$ or $C_5.4H_9.3O_4.2N_9$ (Sawaiker et al., 2012). Hydrogenotrophs that are secreted by extracellular polymers, help to start the granulation process by giving negative charges to bacterial cell and sustain granulation by forming irreversible ECP (Nnaji, 2014).

Generally granule is composed of 28-32% calcium, 18-21% phosphorus, 3-4% magnesium, 2-3% sodium, 0.5-1% potassium and 0.4-0.6% trace elements (Fe, Ni, Co). The process of

stratification occurs in granule with filamentous Methanosaeta Spp forming a core of granule. Predominance of Methanosaeta Spp at the core of granule leads to formation of low VFA concentration while granular core having diverse bacterial population produces high VFAs. Nnaji (2014) reported the Methanosarcina Spp present in granules cores leading to operational problems and it was recommended to initiate the startup phase of UASB with low acetate concentration favoring Methanosaeta Spp.

Granulation rate is determined by the length of startup period of UASB reactor and this rate reduced the sludge washout due to upflow influent regime in UASB reactor. Granulation of granules is highly based on temperature, inert nuclei, multivalent ions (Ca^{+2}, Mg^{+2}, Al^{+3}), wastewater composition, seed sludge nature, pH, alkalinity, organic loading rate (OLR) and hydraulic retention time (HRT) (Nnaji, 2014).

The rate of granulation in UASB reactors determines the length of start-up period and also prevents washout of sludge due to upflow current. Granulation and physicochemical properties of granules are dependent on temperature, inert nuclei, multivalent ions such as Ca^{+2}, Mg^{+2} and Al^{+3}, wastewater composition, nature of seed sludge, essential nutrients, pH, alkalinity and reactor control parameters i.e. hydraulic retention time (HRT), organic loading rate (OLR) (kg COD/m^3.d) and solids loading rate (SLR) (kg COD/kg VSS .d) are important parameters to regulate the granular formation and rapid initial startup of reactor. These parameters define the reactor volume and microorganisms per unit mass present in reactor (Ghangrekar et al., 2005).

Granular sludge stored at 4^{o}C has more structural instability than stored at 24 – 35^{o}C. Due to inhibition of microbial enzymes and lack of substrate availability, the granules are not able to maintain their structure at low temperature which also leads to initial sludge washout. However, the sludge maintained at room temperature show better methanogenic activity. Complete

granulation can be achieved in 60 days after inoculation of UASB reactor with granulated sludge while it takes around 100 days for complete granulation in non-granulated UASB reactor at OLR of 6 kg COD/m^3.d. Maximum granulation (6mm) can achieve in 6 months at OLR of 15 kg COD/m^3.d. By addition of $CaCl_2$, concentration ranges between 100 -300 mg/L granulation can be initiated as ECP tends to bond with divalent cations due to presence of other complexes. This can be accompanied by biogas production (Nnaji, 2014).

Granulation process at the accelerated rate leads to effective removal of COD. But due to accelerated granulation, excessive addition of Al^{+3} and Ca^{+2} may inhibit methane production due to formation of large granules. For preservation of sludge granules, calcium helps granulation by forming precipitates. This also helps to maintain the structural integrity of granules. But excessively large granules can lead to lower COD removal and methanization due to low F/M ratio leading to sludge washout. Specific methanogenic activity (SMA) reduced in long run leading to low specific area of active biomass. This, in long run, may also lead to accumulation of inorganic substances, reducing the biomass efficiency (Nnaji, 2014).

Table 4: Chemical composition of granular sludge

Components	%of dry weight
Ash	10-23
Nitrogen content	11
Protein	35-60
Carbohydrate Total Extracellular polymers	 6-7 1-4
Organic content	90
TOC	41-47

Source: Sung & Dague (1992)

2.4.4 Biosorption Process

It is the uptake or accumulation of chemicals and particulates from wastewater influent by microbial granules. This mechanism is responsible for accumulation of complex constituents and involves adsorption or absorption into various components of microbial cell. This process is used for removal of heavy metal ions, hazardous organic pollutants and hydrocarbons by biomass in granulated sludge. Biosorption mechanism is carried out via biopolymers which are extracellular polysaccharides, pigments or other material in cell wall structure responsible for metabolism, independent binding or biosorption of metal and radionuclide species. This can be carried out via simple ion exchange method or formation of complexes i.e. chelates.

2.5 Anaerobic Biodegradation of Dyes

In any system, it is the anaerobic environment that generates low redox potential (<50mV). This makes the conditions strictly anaerobic that is very crucial for the proper and thorough decolorization of dyes. In literature, decolorization under strict anaerobic conditions is termed as dye reduction. Fundamentally, there is an involvement of biochemistry of structure of azo dyes that are being reduced. The azo bond cleavage –N=N– implies a shift of four electrons (reducing equivalents). At azo linkage, this reaction goes through two stages. Transfer of two electrons take place at each stage to the azo dye, which acts as the final electron acceptor (dos Santos et al. 2007).

$(R1\text{-}N{=}N\text{-}R2) + 2e^- + 2H^+ \longrightarrow R1\text{-}HN\text{-}NH\text{-}R2$ (Hydrazo intermediate formation)

$(R1\text{-}HN\text{-}NH\text{-}R2) + 2e^- + 2H^+ \longrightarrow R1\text{-}NH_2 + R2\text{-}NH_2$ (Reductive cleavage of azo bond)

It has been reported that anaerobic bacteria have the ability to breakdown the azo linkages very efficiently (Wallace et al. 2001). By this reduction, the structure of chromogen completely

changes as well as observed color is completely removed. Research reported that in anaerobic environment, some azo dye intermediates get fully stabilized whereas some are not prone to it (Wallace et al. 2001).

Many studies at laboratory scale indicate that better decolorization of textile effluents with considerable COD removal can be achieved using anaerobic biological treatment technologies. A study was conducted by Lourenco et al., (2001) on SBR with successive anaerobic/aerobic phases. These were used for observing biological color removal from simulated cotton textile effluent that contained azo reactive dye Remazol Brilliant Violet 5R. The level of COD reduced to 30% after 0-50 days of anaerobic sludge acclimatization whereas it removed about 90% from feed dye concentration of 90 mg/L completely in anaerobic phase. This removal of color was accredited to microbial degradation instead of adsorption phenomena. In denitrifying conditions, due to change in SRT, there was a drop in amplified decolorization efficiency. By this, it was also concluded that increasing anaerobic phase did not support decolorization rates anymore. As compared to complex diazo dye, color removal percentage that was obtained for biodegradation of mono azo dye was much higher. Somasiri et al., (2006) demonstrated in one of his study that an anaerobic system has the ability to decolorize three acid dyes completely that belonged to three different chemical groups including Acid Red 131, Acid Blue 204 and Acid Yellow 79. It was observed that under strict anaerobic conditions, by increasing dye concentration from 10 to 300 mg/L at 2000 mg/L COD and HRT of 24 hrs, maximum color and COD removal of 89 and 85% was achieved. VFA and alkalinity in effluent were well suited with anaerobic conditions, showing the stability of reactor. In another study, Manu & Chaudhari (2002) studied the outcome of dye concentration in effluent on microbial action and stated Acid Orange 7 and Reactive Black 3HN can get easily decolorized under anaerobic conditions and achieved color removal

greater than 99% for both dyes at dye concentration of 100 mg/L at HRT of 10 days. However, they concluded that methanogenic activity is inhibited, if the dye concentration is greater than 400 mg/L.

Isik & Sponza (2008) studied that under anaerobic/aerobic treatment, degradation of intermediate metabolites of dyes take place. A simulated textile wastewater containing mixture of 5 dyes (Reactive Black, Direct Red, Direct Black, Direct Brown and Direct Yellow), acetic acid, soluble starch, carboxymethyl cellulose (CMC), glucose, salts, acids and other additives was studied. Moreover, the effect of change of HRT on biodegradation of dyes in anaerobic/ aerobic phase was investigated. It was reported that by combination of anaerobic/aerobic phases, maximum 91% color removal and 84% COD removal is achieved at HRT of 24 hrs. By the reduction of dyes in a very short HRT, colorless aromatic amines can be produced. These colorless aromatic amines did not further metabolize and get accumulated under anaerobic conditions. This study revealed that removal of 85% primary aromatic amines (PAAs) can be done at HRT of 6 days under aerobic conditions only. Another study conducted by Isik & Sponza, (2005a) and Tawfik et al., (2014) investigated the metabolism of total aromatic amines (TAA) under anaerobic conditions and stated that initially, azo dyes were reduced, followed by aromatic amines production and then color removal was achieved. 58% COD, 100% color and 39% TAA (total aromatic amines) removal efficiency was achieved in 100 mg/L of COD as external co-substrate.

Ong et al., (2005a) evaluated the decolorization of Methylene Blue (MB) under anaerobic UASB reactor. The reactor was operated under batch conditions at HRT of 24 hrs. They found that as MB entered the UASB anaerobic reactor, it gets decolorized immediately but it re-oxidized again. This re-oxidization is done by air after getting discharged from reactor and lowers the color removal efficiency. This study imposes the importance of availability of substrate for

effective decolorization as organic content plays as an electron donor/ mediator in biodegradation of dye. Ong et al., (2005b) in his another study used same anaerobic UASB reactor with aerobic SBR system for the treatment of azo dye Orange Ⅱ and showed that with increase in temperature to 30^{o}C and HRT to 48 hrs, respectively, the removal efficiency increases. In UASB system nearly complete decolorization (>95%) was accomplished at dye concentration of 300 mg/L-d and HRT of 24 hrs, whereas aerobic SBR showed insignificant removal of azo dye. It was reported that increase in HRT enhanced the azo dye removal in UASB system as longer HRT provide longer period for microbes to acclimatize to higher dosages of azo dyes.

Firmino et al., (2010) performed a comprehensive study on anaerobic reactor. This study is done by dividing it into two compartments, comprising of acidogenic and methanogenic consortia and compared the color removal efficiencies between the two reactors for Congo Red (CR) dye. It was reported that by applying very high concentration of dye, high removal efficiencies were obtained collectively. But acidogenic reactor played a major role on dye reduction as compared to methanogenic reactor indicating the strong role of fermentative microbes. It also revealed that decrease in HRT from 24 to 12 hrs did not significantly affect the results, showing that electron transfer was not a concern.

2.5.1 Dye Biodegradation under Acidogenic and Methanogenic Phases

Equivalent participation from multiple diverse types of acidogenic, acetogenic and methanogenic bacteria, anaerobic methanogenesis consortia works in synchronized environment with them. As written and marked at varied stages in literature, azo dyes are biochemically reduced by anaerobic sludge consortia, anaerobic sediments and anaerobic enrichment cultures. Dye degradation is because of primary electron donors exclusively under methanogenic conditions.

Acetate and other VFAs entertain as poor or deprived electron donors. However, glucose, ethanol, H_2/CO_2 and formate tend to be operative electron donors for biological dye degradation.

Considering methanogenic environment, extensive literature review directs the part of varied bacterial groups for decolorization of azo dyes. With the chemical transformation capabilities of azo dye, its reduction is a co-metabolic reaction that minimizes equivalents formed as a result of primary complex substrate reduction. Methanogens favors the reducing equivalents designed by fermentative bacteria in order to form methane in an anaerobic bacterial culture. Despite methanization, reducing equivalents for decolorization are used by methanogens. This is why for dye reduction of azo dyes, fermentative bacteria act a pivotal part but more investigations point towards the importance of acidogenic bacteria in dye reduction too.

The rate was reliant on added organic carbon source and simpler dye structure but dye decolorization under anaerobic circumstances cannot be appropriately detailed as most azo dyes with complicated structures were decolorized. Amongst decolorization rate and molecular weight of dyes, no relation was identified that indicated non- specific procedure of decolorization rate. That is why, liable upon scarce varied characteristics, anaerobic azo dyes degradation happens to be an unpredictable process. However, studies indicate that decolorization is the answer to extracellular reaction among reduced compounds produced by anaerobic biomass. Some researchers have evaluated respective relations among anaerobic biodegradability and electrochemical features of azo dyes. It is indicated that elevated decolorization can only be achieved as a result of ORP values dropping below -50 mV (Pandey et al., 2007).

2.5.2 Dye Reduction Mechanism

The simplest procedure of decolorization by anaerobic bacterial consortia is through adsorption of dyes on biomass but this experiment can be compared and contrasted to physical adsorption techniques. Dye is concentrated onto the biomass in the adsorption mechanism, which makes it unsuitable for permanent treatment. This makes it unable to carry on the process sustainably and biomass needs to be disposed of. Biological interface between dye and bacterial cultures is one of the pivotal steps in ultimate reduction of dyes (Pearce et al., 2003).

The cleavage of -N=N- bond in dyes' structure happens to be the foremost step in microbial degradation of dye (aerobic/anaerobic). This reduction undertakes altered mechanisms i.e. low molecular weight redox mediators, chemical biogenic reductants or their amalgamation (Figure 1). Being intracellular or extracellular in reactions (Pandey et al., 2007), azo dyes pertain sulphonate substituent groups and high molecular weight making cell membrane problematic to be passed through. This directs that dye reduction is not due to intracellular dye uptake; on the contrary, it is an extracellular movement incorporating electron transport-linked reduction of dyes. Being impermeable, bacterial cell membranes bound the passage of reducing equivalents from cytoplasm to sulphonated azo dyes. A biochemical reaction arises between intracellular electron transport system and high molecular weight of azo dye molecules in extracellular reduction. For this, electron transport components are better off in outer membrane of bacterial cells (Gram Negative Bacteria). This is where they retort with azo dye substrate or redox mediator at cell surface (Pearce et al., 2003).

As compared to other aerobic conditions, working of reductive breakdown of azo compounds mechanism tends to occur differently. This happens because of diverse redox active compounds (reduced flavins, hydro-quinones) that hastily react with oxygen or accessible azo dyes. Oxygen

and azo dyes contest for reduced electron carriers under aerobic conditions. These reactions follow highly vague reduction processes as they are impulsive. Removal rate of azo dyes increases with the addition of redox mediators to severely anaerobic bacterial consortium. Because of reduction of inorganic compounds, extracellular reduction of azo dyes can also transpire (Fe^{+2}, H_2S). This is the consequence of end products of anaerobic bacterial degradation of azo bonds. Formation of H_2S is due to biodegradation of azo dye Reactive Orange 96 (Stolz, 2001).

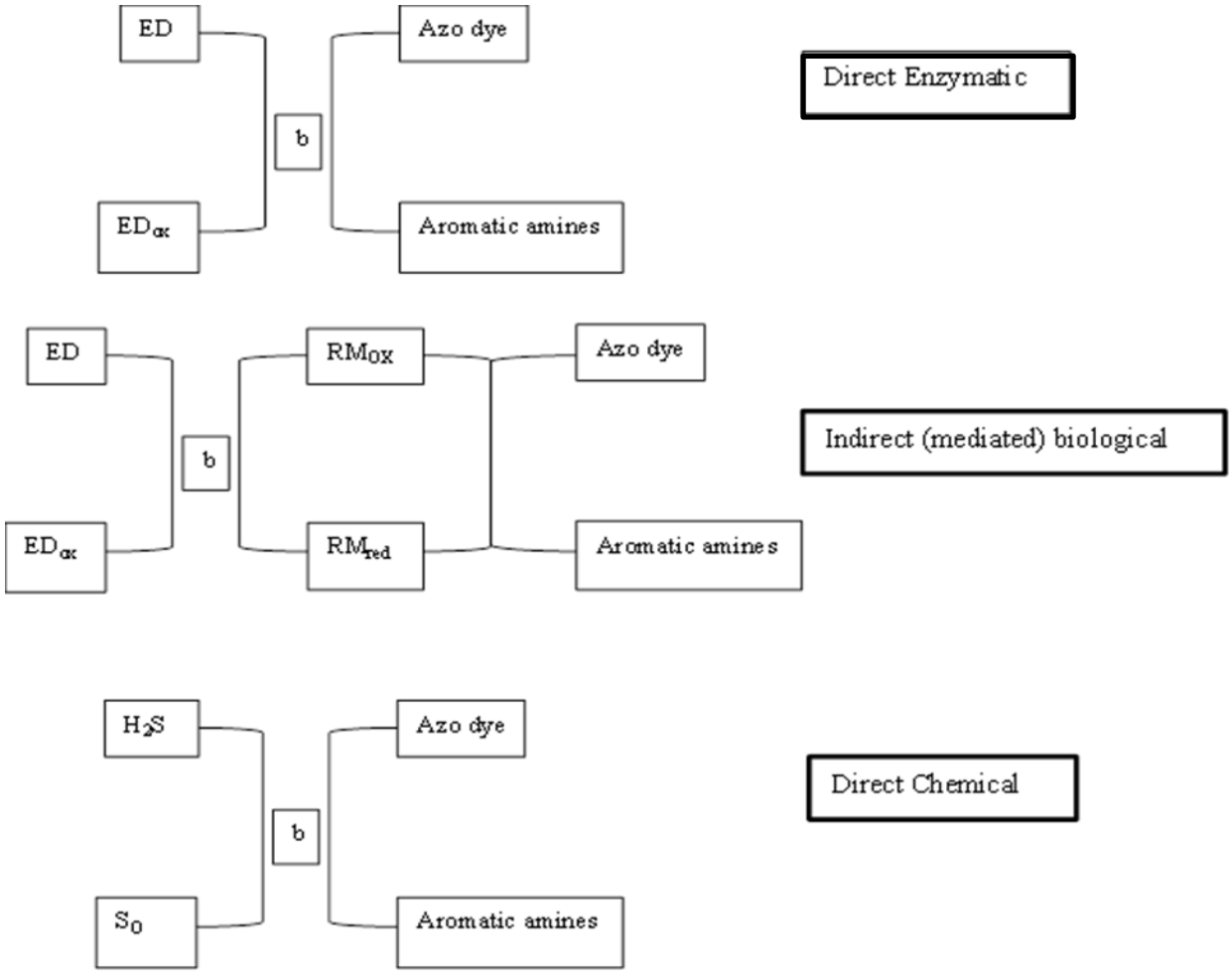


Figure 2: Schematic representation of different mechanisms involve in anaerobic azo dye reduction (Pandey et al. 2007)

- RM =redox mediator, ED =electron donor, b =Bacteria (enzymes)

2.5.3 Biodegradation of Aromatic Amines

Ensuring its thermodynamic stability, primary aromatic amines (PAAs) attract extensive quantities of negative resonance energy. Complicated enzymatic system of microbes especially bacteria is liable to biodegrade the benzene structure under aerobic and anoxic conditions. Initial reduction mechanism encompasses the addition of other functional groups of aromatic ring with hydroxyl group for the period of aerobic biodegradation. This further breakdowns the dye structure by comprising two oxygen atoms. Incorporation of carboxylation, reductive dehydroxylation and other additive reactions are a result of cleavage of aromatic ring under aerobic circumstances and does not occur in aerobic metabolism (Pandey et al., 2007).

Constraining further degradation, diverse researchers explored that biodegradation of azo dyes leads to aromatic amines intermediates under strict anaerobic conditions. Nevertheless in literature, likelihood of complete mineralization of scarce simple aromatic amines is described under acidogenic or fermentative methanogenic circumstances. Other studies discovered that being pretty recalcitrant to aerobic bacterial degradation, aromatic reactive dyes having substituents encompasses nitro and sulphonic groups (dos Santos et al., 2007). When looked into further few extraordinary studies devised that biological degradation of aromatic amines is probable under aerobic and anaerobic circumstances (Couras et al., 2015; dos Santos et al., 2007; Işik & Sponza, 2005a; Muda et al., 2010). Also, it's been testified that colorless aromatic amines pertaining hydroxyl and carboxyl groups are completely mineralized under anaerobic methanogenic conditions (Somasiri et al., 2006).

Table 5: Potential aromatic amines having carcinogenic evidence

Aromatic Amine Group	Human Carcinogen Indication
1-Napthylamine	Mixed
2-Napthylamine	Good
2,5-Diamiotoluene	Slight
3,3'-Dichlorobenzidine	Mixed
3,3'-Dimethoxybenzidine	Mixed
3,3'-Dimethylbenzidine	Slight
4-Biphenylamine	Good
4-Nitrobiphenyl	Slight
4,4'-Methylenebis (2-chloroaniline)	Slight
Auramine	Slight
Benzidine	Good
Magenta	Slight
N-phenyl-2-napthylamine	Some
N,N-Bis(2-chloroethyl)-napthylamine	Good

Source: Wallace et al., (2001)

2.5.4 Factor affecting Dyes biodegradation

Multiple factors could affect the rate of dye biodegradation as many varied types of biological treatment systems are being used in treating textile effluents. Literature has revealed the issues in concern with decolorization rates which are considered to be predictable or unpredictable and can be improved further. The following few factors are explained

2.5.4.1 pH

For effective working of aerobic and anaerobic bacterial cultures, wastewater pH is one of the foremost factors desired to observe. Wallace et al., (2001) testified the effect of wastewater effluent pH on decolorization rates but unfortunately he was incapable of producing the relationship amid two parameters. Nevertheless, an exponential upsurge in dye degradation rate is perceived with decrease in pH. Since this relationship was proven on the particular dye being tested, one cannot count on this statement. No statistical data reports an indirect growth in decolorization rate with decline in pH as what added literature establishes. But the rate of dye degradation is affected by the gigantic decrease or increase in pH range and optimum pH for color exclusion is at neutral or slightly alkaline pH value. This is one the reasons why textile effluents are usually buffered to increase dye degradation rate through bacterial consortia. Bring basic in nature and underwriting a trivial increase in pH, biological reduction of azo dyes yields aromatic amines metabolites (Pearce et al., 2003).

2.5.4.2 Nitrates and Oxygen Requirement

Effect of oxygen for microbial cell growth and dye reduction is a keynote to be considered. Oxygen has important effect on biological features of cells during their cell growth stage. If aerobic reaction occurs during the decolorization of azo dyes, oxygen constrains the dye reduction process except it's a high redox potential electron acceptor. This happens as oxidized electron donors be generated by cells reduce the azo dye than oxygen molecule. But the intermediates of dye metabolites get re-oxidized by molecular oxygen. This indicates that increase in concentration of oxygen in effluent should be eluded as it causes inhibition of azo dye degradation by re-oxidation of colorless intermediates into colored compounds. However, it inclines to be impermanent under aerobic conditions rather it has an irreversible effect.

Reduction ability is given back under anaerobic conditions when pure nitrogen air is substituted with oxygen free nitrogen. Ever since the inhibition of azo degradation enzyme or the favored reduction of oxygen than azo dye metabolites, lethal effect of oxygen on bacterial azo dye degradation is depicted by its detrimental effect on anaerobic bacterial consortia. Additional degradation of dye metabolite may not be possible under stringent anaerobic circumstances. Thus, numerous literature reviews suggest aerobic post treatment as a requisite for degradation of azo dye metabolites. A bi-stage combined anaerobic/aerobic process is commonly recommended for broad mineralization of azo dye intermediates for thorough wastewater treatment (Pearce et al., 2003).

2.5.4.3 Adsorption Mechanism

The key, non-dye related parameter is cell porousness and cell wall adsorption of azo dyes for dye removal from wastewater. The effect of dye adsorption on microbial cell wall defines following results:

1) Freundlich adsorption isotherms depicts the trend for low dye loads per weight of biomass but huge variation occurs with change in dye concentration
2) Reduction of dye after adsorption on cell wall may occur depending on dye structure
3) Adsorption on bacterial cell wall does not hinder the reduction capability of microbes.

However, they indicate that variation possibility with azo dyes and biological dye removal treatments. The dye either is totally minimized by adsorption phenomena, or it gets adsorbed for any further delayed microbial dilapidation. Here, one of the important features to consider is bacterial cell permeability. The above study furthermore specifies how tough it is to eliminate all

dyes off by using whole bacterial cells; rather they can also be successfully condensed by facultative anaerobes and obligate aerobes. This statement points out at the restriction of cell wall permeability used for dye degradation (Wallace et al., 2001).

2.5.4.4 Dyes Structure

In biological dye degradation, the structure of reactive azo dyes plays a momentous part. A few dyes tend to biodegrade quicker than others contingent to the number and placement of azo linkages. The reduction process becomes slower and difficult as the number of azo dye linkages increase. In contrast to four mono-azo and six di-azo dyes, one literature review states that two poly-azo dyes displayed only moderate reduction. The authors testified that poly- azo dyes are hard to decrease as compared to mono- or di-azo dye structures. Solubilizing side groups and nucleophilic reactive groups are encompassed by reactive azo dyes. Complete biodegradation of dyes get difficult as a result of these group features. Type, number and position of these groups are the variables for effective adsorption of dye molecule on granules. Hydrophilic sulfo -groups condense the adsorption of dye molecule. However, in the presence of hydroxyl, nitro and azo groups in dye molecule, adsorption becomes efficient (Wallace et al., 2001).

2.5.4.5 Dye Concentration

Dye removal rate is affected by concentration of initial dye in wastewater. Effort to effectively biodegrade the dye completely is imparted by an approximate toxicity level of dye at high concentration and its capacity to degrade at low concentration by microbial enzymes. As one literature review reports, rate of dye biodegradation lessens after rapid initial reduction of dye. This happens as a result of toxic circumstances interrelated with substrate metabolites fashioned throughout dye reduction. More time is consumed to eliminate the color completely as dye

concentration gets higher. Even with the rate of decreased dye biodegradation, 1-10 mM concentration of dye can easily be decolorized. Nonetheless another literature reported no effect of dye concentration on reduction rates. This consequence is well-suited with non-enzymatic and microbial reduction techniques that are monitored by mechanisms independent of dye concentration (Pearce et al., 2003).

2.5.4.6 Temperature

Color removal efficiency intensifies with increase in temperature in multiple biological treatment systems, succeeding one defined range of temperature operated on the system. The period of maximum color removal is reasonable only when optimum cell growth temperature lies in between 35 -45^{o}C. The reason for color degradation decline rate is loss of cell sustainability or denaturation of cells at high temperatures. However, for a short duration of time even up to 60^{o}C, azo reductase enzyme becomes thermostable for certain bacterial cultures. Microenvironment provides protection to cells in anaerobic systems to stabilize the effect of increase in temperature for maximum color removal rates (Pearce et al., 2003).

2.5.4.7 Glucose Requirement as Substrate

Throughout the reduction of azo dyes bonds leading to decolonization, co-substrate is offered to dyed wastewaters for donation of electrons (Somasiri et al., 2006; Wijetunga et al., 2010). Increasing metabolic activities and granule decolorization rates of microbial granules are readily obtainable for biodegradable carbon source (glucose) (Somasiri et al., 2008). Color removal can be accomplished through biodegradation process, adsorption of dye molecules onto microbial sludge granules and furthermore adsorption followed by biological degradation (Wijetunga et al., 2010).

Higher decolorization rates can be achieved by accumulation of electron donating primary substrates instead of glucose. Oxidation of these substrates creates electrons which are used for the formation of reduced co-factors. It was studied that addition of external carbon sources such as glucose and acetic acid stimulates the azo dyes biodegradation. Dye degradation is supported by endogenous substrate in microbial granules associated with hydrolysis of sludge biomass. Thus, only a small portion of sludge is consumed to supply the required reducing equivalents (Ong et al., 2005a).

2.6 Upflow Anaerobic Sludge Blanket (UASB) Reactor

2.6.1 Microbial Activity in UASB reactor

Upflow anaerobic sludge blanket (UASB) reactor's performance and its effectiveness are highly reliant on the strength and activity of microbial cultures in the reactor. The anaerobic acclimatization and stabilization of wastewater constituents in UASB reactor is a complex and subtle process, which need strict monitoring of anaerobic sludges or anaerobic sediments. This procedure undergoes hydrolytic, acidogenic, acetogenic and methanogenic bacteria. Hydrolysis is a rate limiting, temperature dependent process which occurs at thermophilic temperatures, while acidification is pH dependent process which works best at maximum pH of 6 and mesophilic temperature between 34 and 36^{o}C. Optimum growth of acidogenic bacteria occurs at low pH and short HRT, a condition inhibitory to methanogens. This observation is independent of temperature, as it was reported that acidogenic and methanogenic bacterial activity is 1.6 to 1.8 times faster at thermophilic temperature (55^{o}C) than mesophilic temperature (36^{o}C). Lower HRT, higher disinfection rate and lower viscosity are advantages observed at thermophilic temperatures. But the cost the maintenance of UASB reactors at high temperatures is critical (Nnaji, 2014).

2.6.2 Methanogens Activity in UASB reactors

Methanogens can biodegrade acetate and hydrogen to gaseous by-products at limited pH range of 6 – 8. Methanogens are further classified into acetoclastic methanogens that metabolize methane from acetate; and hydrogenotrophic methanogens that produce methane from hydrogen and CO_2. Acetoclastic methanogens are present in abundant population than hydrogenotrophic methanogens in anaerobic cultures. These acetoclastic classifies as Methanosarcinales (Methanosaetaceae and Methanosarcinaceae) while hydrogenotrophic methanogens are in the orders of Methanobacteriales, Methanomicrobiales and Methanococcales.

Two-thirds or more methane produced in anaerobic reactors is formed from acetate. Among various methanogenic genera, Methanosaeta and Methanosarcina Spp. produce methane from acetate by acetoclastic reaction. Methanosaeta Spp. utilizes only acetate as substrate, can work best at temperature of 37^0C and pH of 7.8, but shows reduced activity at pH below 6.8. Whereas Methanosarcina Spp. forms methane by utilizing different substrates, can stand a wider pH range of 5 -8 and temperatures between 40 – 45^{o}C. Methanosaeta Spp. has large affinity to acetate, about 5-10 times higher than Methanosarcina Spp. having higher growth rate at low acetate concentration. However, at higher acetate concentration, Methanosaeta Spp. is in abundance because of higher pH tolerance range. Methanosaeta Spp. is solely acetoclastic while Methanosarcina Spp. is metabolically versatile as they can form methane from H_2/CO_2 (hydrogenotrophs), from methanol and methylamines (methylotrophs) and from acetate (acetoclasts). Both genera have different morphologies and growth kinetics. Methanosaeta Spp. are slow growing long filaments, sometimes are sheathed rods, with minimum doubling times of 4d under mesophilic conditions. Their high growth rate is due to high affinity for acetate. These

microbes are known as scavengers. In contrast, Methanosarcina Spp. grow at faster rate (minimum doubling time of 1.5d) but have poor affinity for acetate (Figure 2) (Bell, 2002).

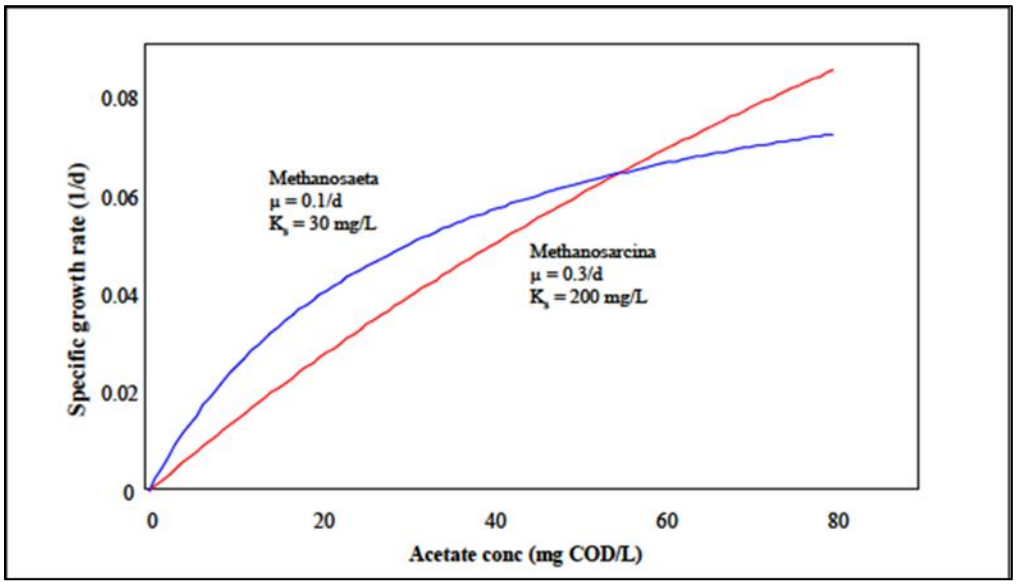


Figure 3: Growth kinetics of Methanosaeta and Methanosarcina (Bell 2002)

Hydrogenotrophic methanogens are more resilient to environmental changes; grow at thermophilic temperatures and short HRT, and can withstand to acidic changes than acetoclastic methanogens. Acetoclastic methanogens are sensitive to NH_3 as well where growth rates of acetoclastic and hydrogenotrophic methanogens are halved at NH_3 concentrations of 4g/L and 7.5mg/L respectively. Hydrogenotrophic methanogens cannot compete with acetogens for utilization of hydrogen as substrate. Homoacetogens completely fail hydrogenotrophic methanogens for hydrogen at low temperatures, initially forming acetate, followed by methane as compared to direct conversion of H_2/CO_2 to methane at high temperatures. Hydrogenotrophic methanogens also excel homoacetogens at lower hydrogen concentration. Homoacetogens are strict anaerobic bacteria that catalyze the formation of acetate from H_2 and CO_2. The environmental conditions favoring the growth of one species may inhibit the existence of other. This competition even exists between different species of same methanogenic group. A slight

imbalance may result in detrimental results for the performance of some species over others. It is necessary to maintain a balanced relationship between acetoclastic and hydrogenotrophic methanogens for effective performance of UASB reactor (Nnaji, 2014).

Table 6: Role of methanogens in anaerobic digestion

Functions Performed	Metabolic Reaction	Process Implication
I. Proton regulation	$CH_3COO^- + H^+ \rightarrow CH_4 + CO_2$	Removal of toxic metabolites and maintain pH
II. Electron regulation	$4H^+ + CO_2 \rightarrow CH_4 + 2H_2O$	Creates satisfactory conditions for breakdown of metabolites, avoids accumulation of some metabolites and increase metabolic rates
III. Nutrient regulation	Excretion of growth factors	Encourages growth of heterotrophs

Source: Ndon (1995)

2.6.3 In-reactor Methanogenic Growth Control Parameter Analysis

Textile effluent can be highly acidified due to possible acidification occurs during process so acidogenic bacterial growth can easily flourished inhibiting the formation of stable granules, causing washout of anaerobic sediments. However, it was been reported in literature that suitable co-relationship must exist between acidogenic and methanogenic bacteria for the formation of granular anaerobic sludge (Singh, 1999). To access the effective performance of anaerobic bacterial culture in granular sediments, few parameter analyses are helpful in strict monitoring of anaerobic reactor performance.

2.6.3.1 Volatile Fatty Acids (VFAs)

UASB reactor performance can get easily suppressed due to excessive accumulation of volatile fatty acids inhibiting the methanogens. VFAs (Acetic acid, propionic acid and butyric acid) together with alcohols, hydrogen and CO_2 make up intermediate metabolites of anaerobic digestion. VFAs are responsible for unpleasant odor from anaerobic reactors. The accretion of VFAs in anaerobic sediments is the indication of incomplete digestion, possibly due to in adequate HRT provided to methanogens to completely biodegrade sCOD, low temperature range or high OLR. It reflects kinetic uncoupling between acid production and consumption, leading to lowering of pH and inhibition of methanogens. The predominant acids in VFAs are acetic, propionic, butyric in UASB reactor, who beyond their concentration range will inhibit the methanogens. The maximum concentration for acetate, butyrate and propionate are 4000, 6000 and 1000 mg/L respectively. Below these concentrations, acetoclastic methanogens increase and above, their properties decline. The buildup of propionate followed by acetate and butyrate has the most crucial impact on UASB reactor performance. Methanosaeta Spp. is predominant methanogen because of ability of organisms to tolerate low acetate concentration. However, accretion of acetate will favor the growth of Methanosarcina Spp. which can tolerate low pH range (Nnaji, 2014).

Though it is reported that 200-400 mg/L as acetic acid of VFAs concentration is normal in literature indicating good anaerobic digestion, the concentration of volatile acids is less important than its rate of time of change into stabilized products (Gómez, 2011).

Table 7: Intermediate volatile acids

Formic acid	$HCOOH$
Acetic acid	CH_3COOH
Propionic acid	CH_3CH_2COOH
Butyric acid	$CH_3CH_2CH_2COOH$
Valeric acid	$CH_3CH_2CH_2CH_2COOH$
Isovaleric acid	$(CH_3)_2CHCH_2COOH$

Source: Ndon (1995)

2.6.3.2 pH and Total Bicarbonate Alkalinity

The relationship between pH, alkalinity and VFAs are the effective measures to completely analyze the anaerobic reaction operational performance. pH can be measured online as pH probes are limited in sensitivity. Measurement and adjustment of pH alone, is not enough as sufficient alkalinity in reactor is required for proper pH control. pH and alkalinity is dependent on chemical equilibrium inside the reactor and affected by many chemical species, the most important is carbon dioxide- bicarbonate equilibrium. The function of pH is equilibrium between CO_2 in biogas and bicarbonates in reactor. HCO_3^- alkalinity and pH change as anaerobic digestion proceeds. HCO_3^- alkalinity is much reliable indicator of anaerobic performance decline than pH and a great deal of results can be inferred by measuring pH and alkalinity in anaerobic reactor (Scampini & Belcher, 2010).

VFAs and alkalinity at bottom and upper parts of anaerobic reactors represent the concentration of organic acids. Their values, if checked against pH, COD removal and methanization, give us clear indication of equilibrium between organics consumption and acid and methane production. VFA/Alkalinity ratio is the easiest way to determine the equilibrium.

1) Below 0.15 shows stable condition as most of organic compounds are converted to organic acids and then to methane.
2) Between 0.15 and 0.2 operations requires careful monitoring as reactor is just a step from being overloaded.
3) Between 0.15 and 0.2, maximum attention must be taken as reactor is almost overcharged where F/M ratio is very high
4) Above 0.25, the reactor gets acidified where substrate is converted to organic acids, not methane (Saleh & Mahmood, 2003).

Methanogenic bacteria consume the acid intermediates rapidly when the anaerobic system is in balance. If methanogens growth is hindered by unfavorable environmental conditions, they will not consume the acid rapidly as they are being produced by acidogens leading to volatile acids accumulation in biomass (Abdelgadir et al., 2014).

2.7 Hydrodynamic Aspects of UASB reactor

2.7.1 Sludge Blanket Height in Reactor

Playing a pivotal part in avoiding sludge washout by regulating solid concentration, an imperative design parameter is UASB reactor sludge blanket height. Typically sludge granulation process, sludge retention and sludge washout is controlled by reactor hydrodynamics. Placed in lower part of reactor, substrate assimilation and gas production is conducted in its sludge bed. Sludge granulation and treatment efficiency gets improved by a tender mixing in sludge bed zone. The gas leakages from bed lifts solid in upward direction and forms a sludge blanket in between sludge bed and GLSS. GLSS diminishes the sludge washout from reactor to sustain a threshold absorption of sludge at blanket top, (Singh, 1999).

2.7.2 Hydraulic Pattern

The flow pattern in sludge bed zone happens to be an additional central factor in design of UASB reactor which smooths its efficient treatment. Mostly, reactor hydrodynamics control sludge granulation process, sludge retention and sludge washout. Substrate assimilation and gas production takes place in sludge bed of reactor placed in lower part of reactor. A gentle mixing in sludge bed zone improves sludge granulation and treatment efficiency. The gas that escapes from bed lifts solid in upward direction and forms a sludge blanket in between sludge bed and GLSS. To maintain a threshold concentration of sludge at blanket top, GLSS minimizes the sludge washout from reactor. A general idea of flow pattern can be achieved through residence time distribution studies using tracer injection methods. However, fluid flow pattern is affected by biogas bubbles, sludge bed depth and aggregated particles in UASB reactor (Singh, 1999).

2.7.3 Soluble Microbial Products

High residual soluble COD (sCOD) in effluent is major treatment in UASB reactors. Not completely degradable, this is due to formation of soluble microbial products (SMPs) in reactors. They are organic compounds fashioned during biotransformation phases of substrate and biomass decay. They comprise of diverse range of low and high molecular weighing compounds such as humic and fulvic acids, polysaccharides, proteins, nucleic acids, organic acids, antibiotics, steroids, enzymes, structural components of cells and metabolic products. The type and amount of SMPs in effluent is a subject to microbial systems type tangled in and their process operating parameters.

2.8 Factors controlling UASB Performance

2.8.1 Upflow Velocity

Upflow velocity is directly proportional to reactor height and inversely proportional to HRT or reactor volume. With the suitable biomass mixture with reactor height, it regulates with or without channeling. The allowed velocity is 0.5-1.5 m/h (Latif et al., 2011). It is important to monitor this parameter as it has settling velocity more than the upflow velocity that is retained in the reactor. Raise in influent flow can increase the bed of sludge that may expand or unsettle with washout of colloids and formation of scum or thick layer on the top of the reactor. This washout of sludge occurs at velocity i.e. greater than 1m/h. However, apparent velocity could be 5-15m/h by increasing sludge loading rate and recycling effluent (Nnaji, 2014). Wastewater flow inside the reactor must follow a strict guideline of velocity throughout the reactor

- Upflow velocity at reactor bottom: 1m/h
- Velocity through GLSS: 3-5 m/h
- Velocity at upper settling area: 1-3 m/h for granulated sludge (Saleh & Mahmood, 2003)

According to some literature, the substrate diffusion rate reduces with high flow velocity; while others deduced that external mass transfer resistance is decreased with high flow velocity. Flow velocity has a particular effect on the thickness of biofilm and its compactness under conditions of turbulent flow, which lead to different mass transfer rates. An increase in upflow velocity decrease acidogenic activity of sludge which leads to VFA accretion for higher loads. The liquid upflow velocity has direct effect on biomass aggregate size in hydrodynamic regime and has little effect on washout of small biomass colloids at higher velocities (Couras et al., 2015).

Table 8: Upflow velocities recommended for UASB reactor design

Influent flow rate	Upflow velocity (m/hrs)
Average flow	0.5-0.7
Maximum flow	<0.9-01.1
Temporary peak flows	<1.5

Source: Uldal (2008)

2.8.2 Mixing

Mixing carries out efficient contact between microbes and substrate and diminishes the mass transfer resistance, lowers inhibitory intermediate buildup and stabilizes environmental conditions. Failure to efficient mixing harms the whole process by accumulating the material at different digestion stages where every stage has different pH and temperature (Latif et al., 2011). It also encourages attachment between biomass and substrate. Mixing is also supported by effluent recirculation, biomass recycling and mechanical fixing that increases the biomass-substrate contact and initiates dead zones in sludge bed that reduces the channeling of influent through sludge (Powar, Kore, Kore, & Kulkarni, 2013). UASB reactor follows completely mixed flow reactor (CSTR) approach where turbulence is created by the ascending flow of gases, but this is not pertinent to UASB reactors as they treat at low strength wastewater. For this, internal mixing is not needed when the operating temperature is low (<20°C) due to high liquid viscosity and slight biogas generation. Poor generation of biogas as a result of inadequate mixing can lead to operational problems at early working stages of UASB reactor (Nnaji, 2014). Mixing in reactor can also be caused by rising biogas bubbles and upflow velocity. Mixing in UASB sludge bed takes shear forces resulting in formation, stability and structure of granules. Small biogas

bubbles adhere to granules and cause the granules to remain in suspended position in sludge bed of UASB reactors (Gómez, 2011).

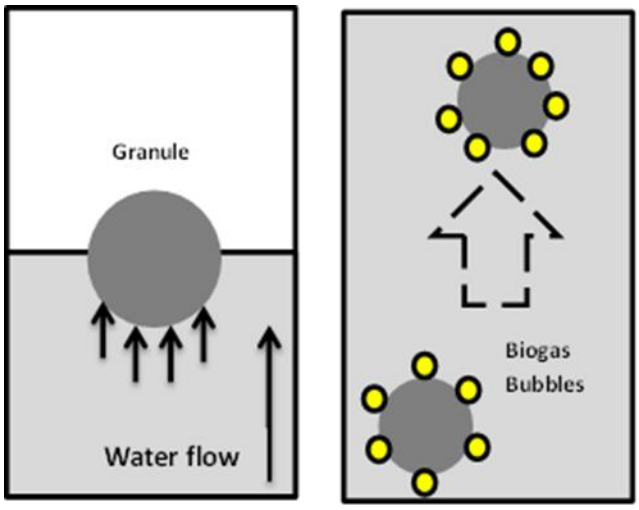


Figure 4: Granules rising due to upflow velocity and biogas attachment (R. Gómez 2011)

When these biogas bubbles get attached to the granules, it can induce adequate upflow velocity along with granule to granule collision that result in agitation in UASB reactor. These phenomena can be varied by particle density, biogas entrapped inside granule or settling, being pushed or dragged due to upflow of velocity or biogas bubbles. This results in granule buoyancy. Granule floating due to these buoyant forces trap bubbles in hollow spaces and with their large cavities at core depicts biogas generation inside a granule (Gómez, 2011).

Repetitive vigorous mixing also known as recurrent mixing is destructive as methanogens are less proficient with constant turbulence being created. Hence it is not recommended to mix the sludge bed during the initial phase, as the pH of digester is low that can lead to reactor instability (Latif et al., 2011). Long HRT is linked to low upflow velocity which is favorable to dispersed bacterial growth but less promising for granule formation.

Contrary, shorter HRT relates to high upflow velocity which leads to non-granulated microbes' washout but promotes speedy sludge granulation. This transforms the flocculent sludge into granules by regulating hydraulic stress which settles these granules (Gómez, 2011). Due to low biogas bubbles, insufficient mass is being generated between substrate and granule occurs. Slow mixing can prevent mass transfer and channeling into the sludge bed, but it increases the operating cost of the reactor.

2.8.3 Hydraulic Retention Time (HRT)

HRT is another principle parameter that monitors the average time of influent residence in reactor before it's discharged with low chances of short circuiting. Acceptable HRT is needed to confirm the adequate time for microbes to degrade organics. HRT in USAB reactor can range from 2-200hrs depending on the type of wastewater and scale of treatment facility. Startup HRT is normally extended than the operational HRT for USAB reactor as it requires more time to acclimatize and prevent flocculent sludge washout. The usual lab scale UASB reactor startup HRT is 2-3 days. As the bacterial culture gets accustomed to wash water nature, HRT is deducted by raising OLR step wise until stability is reached. Elongated HRT will constrain the reactor's performance as it starves the microbial culture, while short HRT restricts the transfer rate and sludge washout. USAB's HRT is designed to retain equilibrium between accessibility of substrate and amount of microbes to consume it i.e. F/M ratio (Nnaji, 2014).

2.8.4 Organic Loading Rate (OLR)

OLR affects considerably the ecosystem of microbial cultures and operational performance of USAB reactor. OLR is the rate at which substrate enters the reactor and is expressed as g of COD/BOD per reactor volume, which may be different from the rate of microbial interaction

with substrate (Ndon, 1995). Different studies has estimated that high OLR will decrease the COD removal efficiency as more substrate will get in contact with lesser microbes with limited time between them. However, gas production increases with high OLR until methanogens are unable to quickly convert the VFAs to methane. OLR can relay to substrate concentration and HRT, and a realistic balance is essential between these parameters (Latif et al., 2011).

2.8.5 Sludge Retention Time (SRT)

The significance of suspended solids and their amount cannot be ignored as it is related to the accumulation of MLSS in the reactor with long SRT stabilized and organics removed. Excessive solids in effluent result in loss of MLSS and short SRT. The incomplete transformation of microbes into intermediate acids is due to microbes' washout in terms of MLSS in effluent, which occurs when there is low SRT as compared to regeneration rate of microbes, resulting in imbalance in microbial community. For aerobic treatment, 50% of substrate conversion into methane and regeneration rate can be achieved which is low for anaerobic treatment where only 15% of substrate removed is converted into new biomass. Thus, high retention of MLSS in UASB reactors is important. SRT is defined as:

SRT (ω_C) = $\frac{X_T}{\Delta X / (\Delta t)_T}$ where,

SRT (ω_C) = Solids retention time, day

X_T = Total active biomass (MLVSS) in the reactor, mg/L

(ΔX) = Daily amount of active biomass withdrawn from the system which includes solids purposely wasted plus those lost in the effluent

$(\Delta t)_T$ = Time interval for biomass wastage, time

A study proposed SRT for anaerobic treatment systems at different temperatures. He conveyed that efficiency of treatment gets low and poor at minimum SRT (Ndon, 1995).

Microbes' growth and SRT are related as $\frac{1}{m} = \mu$ (SRT) where μ is the specific bacterial growth rate (day^{-1}). Lowest SRT is to be maintained to avoid the flocculent bacterial washout and their growth rate can surpass the decay rate. Larger volumes and associated capital cost can meet this condition. New anaerobic technologies integrate the differences where SRT and HRT can be monitored individually. Generally, the granules in reactor are allowed to settle or recycled back to sludge bed by effluent recirculation. This feature of UASB reactor allows long SRTs to be kept at short HRT which reduces the reactor volume and capital cost as well. Recirculation of granules helps in rapid bacterial growth, improves process instrumentation and better reactor stability with less fluctuation (Christensen & Gerick, 2015). Since anaerobic systems have low methanogenic growth rate, large SRT is required to synthesize new cells from degraded organics (Latif et al., 2011).

2.8.6 Food to Microorganisms Ratio

This ratio is substrate load applied to reactor per unit of biomass in reactor and is given as

$\frac{F}{M} = \frac{S_o}{\theta X}$ Where,

F/M = Food-to-microorganism ratio, 1/day

S_o = Influent BOD or COD concentration, mg/L (g/m^3).

Θ = Hydraulic detention time, day

X = Concentration of MLVSS, mg/L

V = volume of reactor, m^3 or L (gallons).

Q = Influent wastewater flow rate m^3/day.

Concluding into minimum efficiency elevated F/M ratio indicates that MLVSS is saturated with food. However, a low F/M ratio illustrates MLVSS undergoes ample degradation of substrate and are starved. High F/M ratio is associated with short SRT whereas low F/M ratio corresponded to long SRT making 0.1 to 1 F/M ratio range operational for efficient treatment.

2.8.7 Nutrients

For the granulation process and control of UASB reactor, prerequisite of ions in feed is critical. Micronutrients (nitrogen, phosphorus, potassium, sulfur, calcium, magnesium) and trace elements (iron, nickel, cobalt, zinc, manganese, copper) require bacteria in anaerobic digestion for optimal activity. Deficiency of nutrients puts a severe effect on bacterial growth even when minute concentration of these elements is needed. These nutrients are not accessible in adequate amounts in numerous wastewater streams i.e. agro-industrial, textile, and pharmaceutical etc. where these wastewater has to be supplemented with trace element prior to anaerobic treatment. The required COD: N: P ratio for enhanced methane yield has been reported as 100: 2.5: 0.5 where macro and micronutrients concentration can be calculated based on BOD/bCOD of wastewater, cell yield and nutrient requirement for bacteria (Rajeshwari et al., 2000).

3 MATERIALS AND METHODS

Details of materials and methods used during this research are briefly explained in this chapter. This include reactor design and operational procedure, feed solutions, sample preparation, analysis techniques performed and equipment specification used.

3.5 Feed Solutions

3.5.1 Real Textile Wastewater Characterization

Real untreated textile wastewater was obtained from Koh-e-Noor Mills Rawalpindi, Pakistan. Six samples were collected; three times a week for 2 weeks and the samples were characterized for specific parameters for synthetic duplication of real textile wastewater (RTWW) to narrow the range of variability. The samples were analyzed for COD, phosphates, nitrates, halogens, TDS, electrical conductivity, turbidity and alkalinity using standard methods for analytical measurements. The mean values of parameters are given in the table below

Table 9: Characterization of Koh -e-Noor textile mill effluent

Parameters analyzed	Average values *
COD (mg/L)	2540
Orthophosphates (mg/L)	22.27
Nitrogen-ammonia (mg/L)	26.31
Chlorides (mg/L)	745.50
TDS (mg/L)	1870
EC (mS/cm)	3.27
Turbidity	371.50
pH	5.35

*N= no of samples = 6

3.5.2 Synthetic Wastewater Preparation

Synthetic textile wastewater was prepared duplicating the average values obtained from the samples tested from real textile effluent. Single dye instead of mixture of dyes was used initially to optimize the most suitable intermittent phase operated by UASB reactor. Then, synthetic wastewater containing mixture of dyes was being tested at the optimal condition. All laboratory synthetic feeds were prepared using fresh tap water, dyes, major organics and trace additives. The synthetic feed is stable and soluble in water. The COD: N: P ratio of feed is 100: 10: 1 which is essential for biomass growth.

The synthetic substrate was prepared everyday where concentrated synthetic feed of 125 mL was diluted in 12 L of tap water with concentration of COD varying from 2500 to 2150 mg/L. Addition of sodium bicarbonate ($NaHCO_3$) was done to maintain suitable buffering in reactor. Usually 0.5g of $NaHCO_3$ as $CaCO_3$, per 2g of COD was added. Three trace elements were added in feed according to Metcalf & Eddy, (2003).

Table 10: Simulated textile wastewater composition

Dextrose (glucose)	$C_6H_{12}O_6$	2 g
Ammonium Chloride	NH_4Cl	0.38 g
Potassium di hydrogen phosphate	KH_2PO_4	0.109 g
Magnesium sulfate	$MgSO_4.7H_2O$	10 mg
Calcium chloride	$CaCl_2$ granular	20 mg
Sodium bicarbonate	$NaHCO_3$	0.5 g
Ferric chloride	$FeCl_3$ anhydrous	5 mg
Zinc chloride	$ZnCl_2$	1 mg/L of reactor volume
Cobalt chloride	$CoCl_2.6H_2O$	1 mg/L of reactor volume

To optimize the intermittent (non-feeding) period of UASB reactor, the synthetic laboratory feed was fed with single dye (Reactive Bezektiv Orange Cosmos SC) with minimal dye concentration of 10 mg/L to acclimatize the anaerobic sludge to the respective dye characteristics. This synthetic wastewater was the replication of mono dye textile wastewater. Then, two other dyes were used in the laboratory feed giving mixture of dye containing synthetic wastewater and used at optimal intermittent period of UASB reactor. Each dye was measured for maximum absorbance wavelength (λ_{max}) and COD concentration. Each dye was tested for COD contribution in feed using closed reflux colorimetric method. The characteristic wavelength of simulated dye wastewater was determined by running a scan of dye stock solution (10 mg/L for single dye and 30 mg/L for dyes mixture) on UV-Vis spectrophotometer (T-60 U PG Instruments, UK). Dyes used in the study are listed below and are referenced according to their proprietary names.

Table 11: Dyes used during experimentation study

Dye used	Nature of Dye	Manufacturers
Bezektiv Cosmos Orange	Reactive	Bezema
Foron Red S3BS	Disperse	Archrome
Novasol Navy DB	Vat	Hunstman

The maximum absorbance wavelength and formulas for the concentration of dyes in effluent were developed using UV-Vis Spectrophotometer software and MS Office Excel. The concentration of Bezektiv Orange Cosmos dye in effluent was measured by using the formula obtained from the linear curve (Eq. 1) which is

Y (absorbance) = 0.03 x (concentration) – 0.008

Color removal efficiency was determined using Eq. 2

Decolorization Efficiency = [(Cb – Ct)/Cb] x 100

Where C_b and C_t are the concentrations of dye before and after the treatment process.

The initial pH of synthetic wastewater was adjusted to 7 ± 0.5. The synthetic textile wastewater was prepared by mixing stock dye solution in nutrient medium containing nutrients and glucose which contributed for final color of TWW.

3.6 Sample Preparation and Preservation

Mostly the samples are analyzed immediately after being withdrawn from reactor; however, sometimes samples are preserved according to Standard Methods (APHA/AWWA/WEF, 2012). Except for some preliminary testing, all samples are being prepared as follows. Samples with high solids contents were centrifuged at 2000 rpm for 20 minutes and then the supernatant was filtered through Wattsman filter paper #1. The filtrate was collected in clean container and further prepared based for the analysis of COD and color analysis. For TDS, filter paper (ϕ= 47 mm, pore size 2 micron) made of glass fiber was used in vacuum filter assembly and supernatant was used for TDS analysis. This ensured uniform and accurate measurement among all effluent samples.

3.7 Sample Analysis

To determine the reactor performance, several parameters are frequently analyzed in laboratory. The type and frequency of data collection is described in table below. Some parameters are analyzed on daily basis on completion of a cycle and some are analyzed when UASB reactor system had reached steady state. Steady state is achieved when the COD and color removal rates

are within ±10% variable range. Effluent samples were analyzed for COD, color, TSS, VSS, alkalinity, TKN and orthophosphates and sludge sample were analyzed for MLSS and MLVSS in accordance to Standard Methods (APHA/AWWA/WEF, 2012) using procedures 5220C, 2120C, 2540D, 2540E, 2320B, 4500-Norg C and 4500-P C.

COD of effluent sample was determined using closed reflux colorimetric method using COD digestor which digests the COD viols at 120 – 150^{o}C temperature. The COD viols were prepared using H_2SO_4 and $K_2Cr_2O_4$ reagents and 2.5 ml of sample and then digested viols were cooled to room temperature and titrated against ferrous ammonium sulfate (FAS) solution to get the COD concentration in effluent. The samples were tested for soluble COD (sCOD) where the raw sample was filter through GF/C filter paper # 1 using a glass funnel and tripod stand.

Color was measured using the prepared sample (5ml) directly in cuvettes (made of quartz) used for color analysis in UV-Vis spectrophotometer at maximum wavelength and the absorbance value was used in straight line formula to get the concentration of residual dye in effluent. TSS, VSS, MLSS and MLVSS (10ml sample each) were measured according to Standard Methods using filter paper in vacuum filter and the supernatant was used for TSS and MLSS while putting in oven for 1 hr at 105^{o}C and then in muffle furnace for 30 min at 550^{o}C.

VFA and Alkalinity were titrimetrically determined using 0.02N H_2SO_4 solution for 20 ml effluent sample for determination of alkalinity and 0.1N NaOH for determination of VFA in effluent. These parameters are determined once a week whose accuracy is dependent on sample characteristics and is done to estimate the reactor efficiency. If VSS/TSS ratio is between 0.4-0.6, there is no need of centrifugation or sedimentation to remove relatively low amount of solids in sample.

pH was determined using pH meter (OAKTON 300 series) using glass pH probe which is always dipped in 0.1M KCl solution. pH probe was calibrated once a week using 4.00, 7.00 and 10.00 buffer solutions. pH measurements are made as soon as samples are withdrawn from reactor with little or no agitation to minimize the loss of carbon dioxide. Frequent pH measurements were done to monitor the efficiency of reactor system, because changes in pH value towards the acidic range indicates potential imbalance of methanogenic or facultative acidogenic bacterial activity. A sharp drop in pH also indicates the accretion of volatile acids and less alkalinity production hampering buffer capacity of reactor.

TKN (Total Kjeldahl Nitrogen) was measured using the Kjeldahl apparatus, using 25 ml of prepared sample, adding 50 ml of distilled water and 12.5 ml of digestion reagent with glass beads and put to digestion chamber at 300 -400^{o}C. After cooling the sample, pH is adjusted in alkaline range using NaOH-$Na_2S_2O_3$ reagent. Dilute the sample with distilled water and add phenolphthalein indicator. Place the sample in distillation stand and connect with condenser whose tip is inserted in 50 ml of boric acid solution. As the ammonia gets absorbed in boric acid, the solution changes its color. Titrate against 0.02N of H_2SO_4 acid for quantifying the amount of ammonia in effluent sample

Orthophosphate concentration was measured by adding 1 ml of Vanadate-molybdate reagent in 5 ml of prepared sample and measures the concentration using UV-Vis spectrophotometer at 470nm absorbance.

Table 12: Summary of parameters analyzed during experimental study

Parameter	Method
pH	pH meter
Total Alkalinity	*Standard Methods*
Volatile acids	Standard Methods
Chemical Oxygen Demand (COD)	Standard Methods
Color	UV-Vis Spectrophotometer
Solids SS (MLSS.TSS) VS (MLVSS, TSVS)	Standard Methods
Orthophosphates	UV-Vis Spectrophotometer
TKN,TN	Kjeldahl Digestion

3.8 Start-up and Acclimatization Phase

Aerobic sludge was obtained from full scale membrane bioreactor (MBR) plant. This aerobic sludge was blended with 0.5 L of fresh cow dung as seed sludge, 2g of glucose and 0.5g of $NaHCO_3$. The sludge was mixed and put in incubator at 35^oC. Scum, live worms and dead microbes were removed at 48 hrs HRT with effluent discharge and addition of fresh feed. The sludge was placed in air tight jars and flushed with nitrogen gas N_2 to remove oxygen bubbles to maintain strict anaerobic conditions. The reactor content was mixed during last 5 minutes of flushing to enhance the removal of dissolved oxygen. The reactor was mixed daily for complete substrate and microbial connection. After 3 months of continuous batch study, analyzing the COD removal rate reached to steady state of 85%, excessive amount of biogas bubbles production and minimal scum generation, the sludge was transferred to reactor for continuous

operation at 24 hrs HRT with introduction of single reactive Bezektiv Cosmos Orange dye at 10 mg/L concentration.

3.9 Sludge Analysis

The laboratory analysis was carried for sludge inoculum used in UASB reactor system and the fresh sludge was studied for MLSS, MLVSS, VSS, TSS and pH. The measurements performed and sludge characteristics are described in table below

Table 13: Sludge characterization parameters

Parameters	Method/Equipment	Values
pH	pH meter	7.5
Temperature	Thermometer	35°C
TSS (mg/L)	Standard Methods	500 ± 75
VSS (mg/L)	Standard Methods	360 ± 100
VSS/TSS	Standard Methods	0.72
MLSS (mg/L)	Standard Methods	4400 ± 990
MLVSS/MLSS (mg/L)	Standard Methods	0.43

3.10 Experimental Design and Setup

The experimental study was conducted in Water and Wastewater Lab in Institute of Environmental Sciences and Engineering, NUST, Pakistan by setting up a laboratory scale UASB reactor with working volume of 10.87 L. It was fed with synthetic textile wastewater feed prepared using macro and micro nutrients and dyes obtained from Koh-e-Noor Textile Mills, Rawalpindi, Pakistan.

3.10.1 Reactor Configuration and Fabrication

One UASB reactor was used for conducting the research. The dimensions and descriptive schematic diagram of reactor section and plan view is shown in Figure 4 & 5. The reactor was constructed by Acrylic Arts, Lahore under the engineering supervision of 3W systems, Lahore using acrylic sheet and were cylindrical in shape with cone shape bottom. The reactor was 24.4 inches in height and 6.2 in internal diameter. The total volume of reactor was 10.87 L of which 9.978 L was working volume and the remaining 0.89 L was occupied by GLSS in head space of reactor.

Eight sampling ports were installed at 100 mm interval along the length, from the bottom of reactor for feeding, wasting and sampling. Reactor had two other ports above the hopper at top of reactor at 50 mm interval installed in opposite directions. The top most was effluent port while the other below was used for effluent recirculation. All the ports were 7 cm (2.75 inches) long with 1 cm (0.4 inches) internal diameter and were made of stainless steel tubes. The ports were reinforced by stainless steel connectors, ball valves and threaded nipples.

The influent port of 2.75 inches length and 0.4 inches internal diameter was steel-clad with isolation valve placed vertically at the bottom of reactor. The influent enters in the reactor from the bottom conical shape funnel with upper diameter of 52 mm. The top of reactor was fitted with plate having same outside diameter of 230 mm (9 inches) as blind flange of reactor. The plate and blind flange was 0.4 and 0.5 inches thick respectively. The blind flange has eight, 0.39 inches holes in which stainless steel butterfly bolt and nuts were used to fasten the reactor into single reactor. A 0.12 inch thick rubber gasket was used in between flange and top plate to ensure air and leak proof seal.

The top plate of reactor has three holes. The middle hole has diameter of 0.47 inches containing a port reinforced with stainless steel connectors, ball valve and thread nipples. This hole is further connected to an acrylic dome used as Gas-Liquid-solid separator inside the reactor. The bottom of funnel shape dome has diameter of 100 mm (4 inches) taking 4.4 inches length from the top of reactor. The dome is curved at 45^{o} leaving a space of 25 mm from the walls of reactor. Below GLSS dome is the 0.98 inches thick cylindrical hopper made of acrylic sheet is tilted at 45^{o}. This is used for the retention of scum or suspended solids from escaping into the effluent. The other two holes on either side of middle hole on top plate are pressure relief valves to release the pressure exerted by the production of biogas.

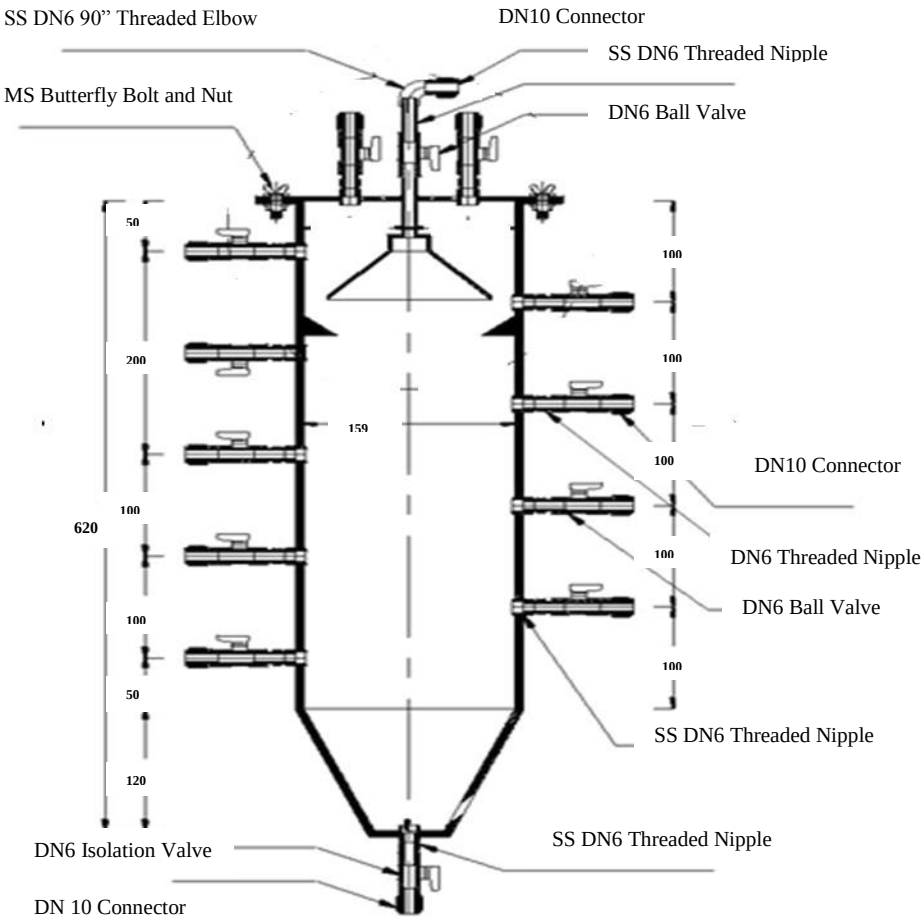


Figure 5: Cross sectional view of UASB reactor

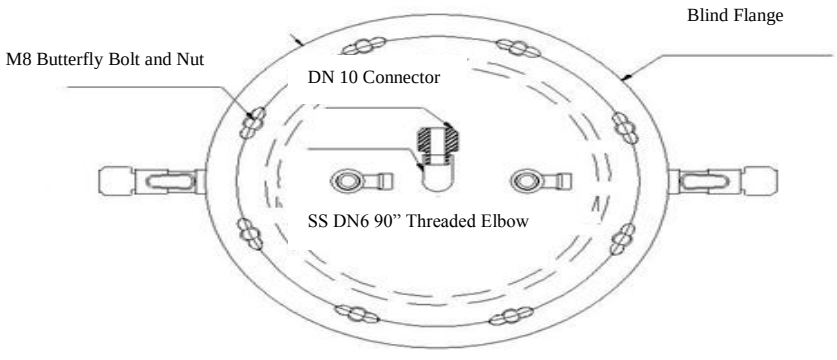
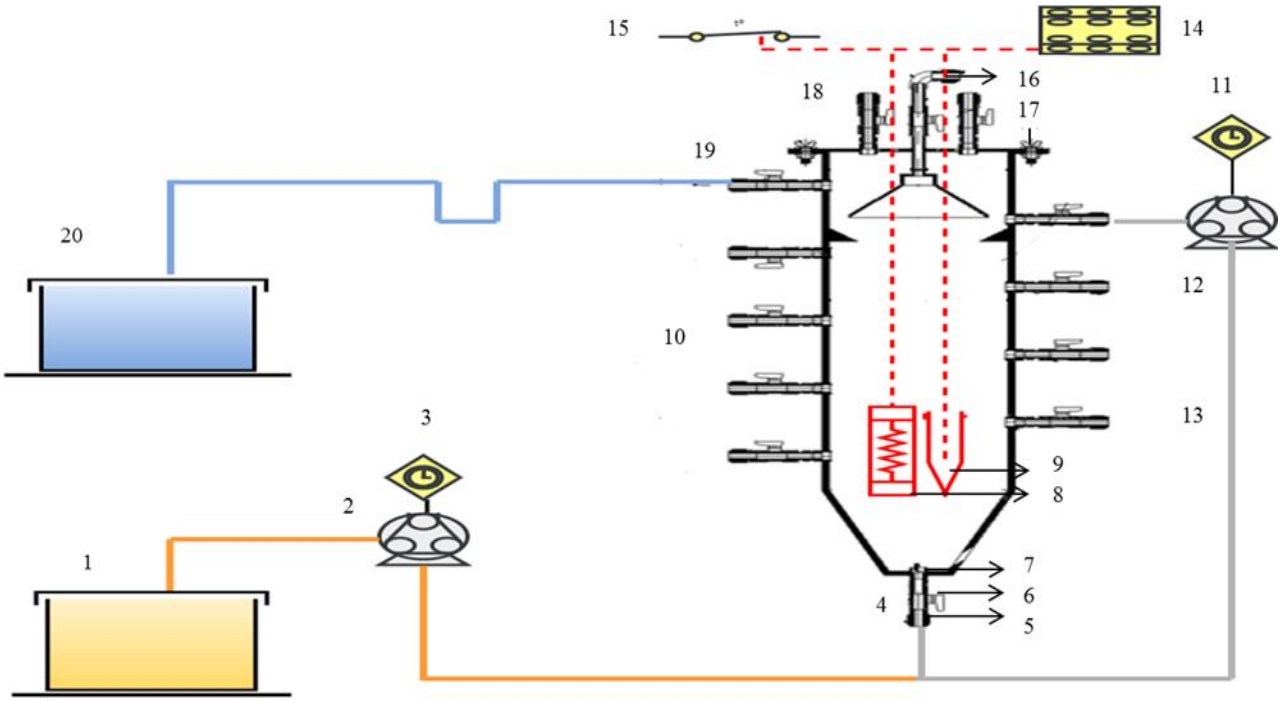


Figure 6: Top view of UASB reactor

3.10.2 Reactor Installation

Upon completion of reactor fabrication, the volumes of reactor were calibrated using measured amount of tap water. Reactor is installed at the proposed station where reactor is clamped on the iron made stand clamped from top and bottom and fixed at placed using steel nut and bolts. The influent is introduced from bottom of reactor where influent is pumped from influent tank. Peristaltic Influent pump (IP) (Longer Pump BT 300 -2J) is placed at suitable head behind the reactor on steel shelves constructed for organized placement of pumps. The top most port is used for effluent discharge and sampling port while the port next to it is used for effluent recirculation. The effluent is recirculated using peristaltic effluent recirculation pump (ERP) (Masterflex L/S Cole- Parmer Instrument Company) from port at the top of reactor to influent port. The pumps operation is regulated using automatic digital timers (DH48S-S), one for influent pump (IP) and other for effluent recirculation pump (ERP) used to regulate the peristaltic pumps at predetermined intervals and effluent recirculation was done throughout the experimental work. Temperature was regulated by heating system containing heating rod, thermocouple, thermostat and magnetic contractor. The piping used in reactor is 1 cm in diameter for influent and effluent recirculation is fitted with connector and Teflon tapes to prevent leakage. Fig shows the experimental set-up of UASB reactor system used in research at laboratory scale.



1. Influent tank
2. Peristaltic feed pump
3. Timer 1
4. Influent port
5. Connector
6. Isolation valve
7. Threaded nipple
8. Heating rod
9. Thermocouple
10. Sampling ports
11. Timer 2
12. Peristaltic effluent recirculation pump
13. Effluent recirculation pathway
14. Magnetic contractor
15. Thermostat
16. Threaded elbow
17. Butterfly bolt and nut
18. Pressure relief valve
19. Effluent port
20. Effluent tank

Figure 7: Schematic configuration of lab-scale UASB reactor

3.11 Reactor Operation

The reactor was seeded with semi flocculent granular anaerobic sludge which was acclimatized from aerobic sludge obtained from full scale membrane bioreactor (MBR) plant installed at NUST, Islamabad and was initially operated at HRT of 24 hrs and OLR of 2 kg/m^3-d using single reactive Bezektiv Cosmos Orange Dye at 10 mg/L concentration. The detail of experimental conditions carried out at UASB reactor system is summarized below in Table

Table 14: Experimental conditions for UASB reactor operation

Single dye feed at 2 kg COD/m^3-d OLR, 24 hrs HRT and 10 mg/L dye concentration (Phase 1)	
Operating Conditions	**OLR (kg COD/m^3-d) during continues cycle**
Continuous cycle (24 hrs)	2
Continuous cycle (18 hrs) Intermittent cycle (6 hrs)	2.67
Continuous cycle (12 hrs) Intermittent cycle (12 hrs)	4
Continuous cycle (6 hrs) Intermittent cycle (18 hrs)	8
Continuous cycle (12 hrs) Intermittent cycle (12 hrs) Continuous cycle (12 hrs) Intermittent cycle (12 hrs)	2 2
Mixed dyes feed at 2 kg COD/m^3-d OLR, 24 hrs HRT and 30 mg/L dye concentration (Phase 2)	
Continuous cycle (18 hrs) Intermittent cycle (6 hrs)	2.67
Continuous cycle (12 hrs) Intermittent cycle (12 hrs)	4
Mixed dyes feed at 2 kg COD/m^3-d OLR, 48 hrs HRT and 50 mg/L dye concentration (Phase 3)	
Continuous cycle (12 hrs) Intermittent cycle (12 hrs) Continuous cycle (12 hrs) Intermittent cycle (12 hrs)	1 1

4 RESULTS AND DISCUSSION

4.5 Optimization of Different Intermittent/Continuous Combinations

4.5.1 COD and Color Removal Rates

A total load of 2 kg COD/m^3-d was applied for each continuous (feeding) and intermittent (non-feeding) combination at 24 hrs HRT and a minimum dye concentration of 10 mg/L. This was done to optimize the adequate non-feeing (intermittent) period provided during the continuous operation of reactor for maximum COD and color removal rates. The length of each run was dependent on biomass adaptation to complex substrate in the feed and sludge characteristics. Effluent recirculation was used in reactor to maintain the reactor's hydrodynamic conditions and maximum adsorption of complex dyes onto biomass granules to achieve complete contact between them. The effluent was recirculated in adjustment with influent flow for each operating run.

Initially the reactor was operated with continuous feeding for 24 hrs HRT at input OLR of 2 kg COD/m^3-d (Run 1). COD and color removal efficiency of 46.70 and 58.69% was achieved (Figure 8). High COD concentration in effluent (1281.68 mg/L) was due to large suspended solids concentration in non-granulated anoxic sludge with low retention capacity. Gradual formation of granulated sludge leads to decrease in effluent SS concentration with improved removal rates. The delay in achieving maximum removal rates in newly installed lab scale UASB reactor demanded time for acclimatization of sludge to substrate. Initially, due to low acclimatization of sludge to influent substrate, the steady state was maintained for a week at low COD and color removal rates. Higher COD and color removal was not achieved though

continuous effluent recirculation was applied. This indicated that mass transfer is not a limiting factor for dyed textile wastewater. Recirculation of effluent keeps on recirculating the solubilized material in reactor, keeping the COD and dye residual concentration stable and high. This had no considerable effect on reactor process.

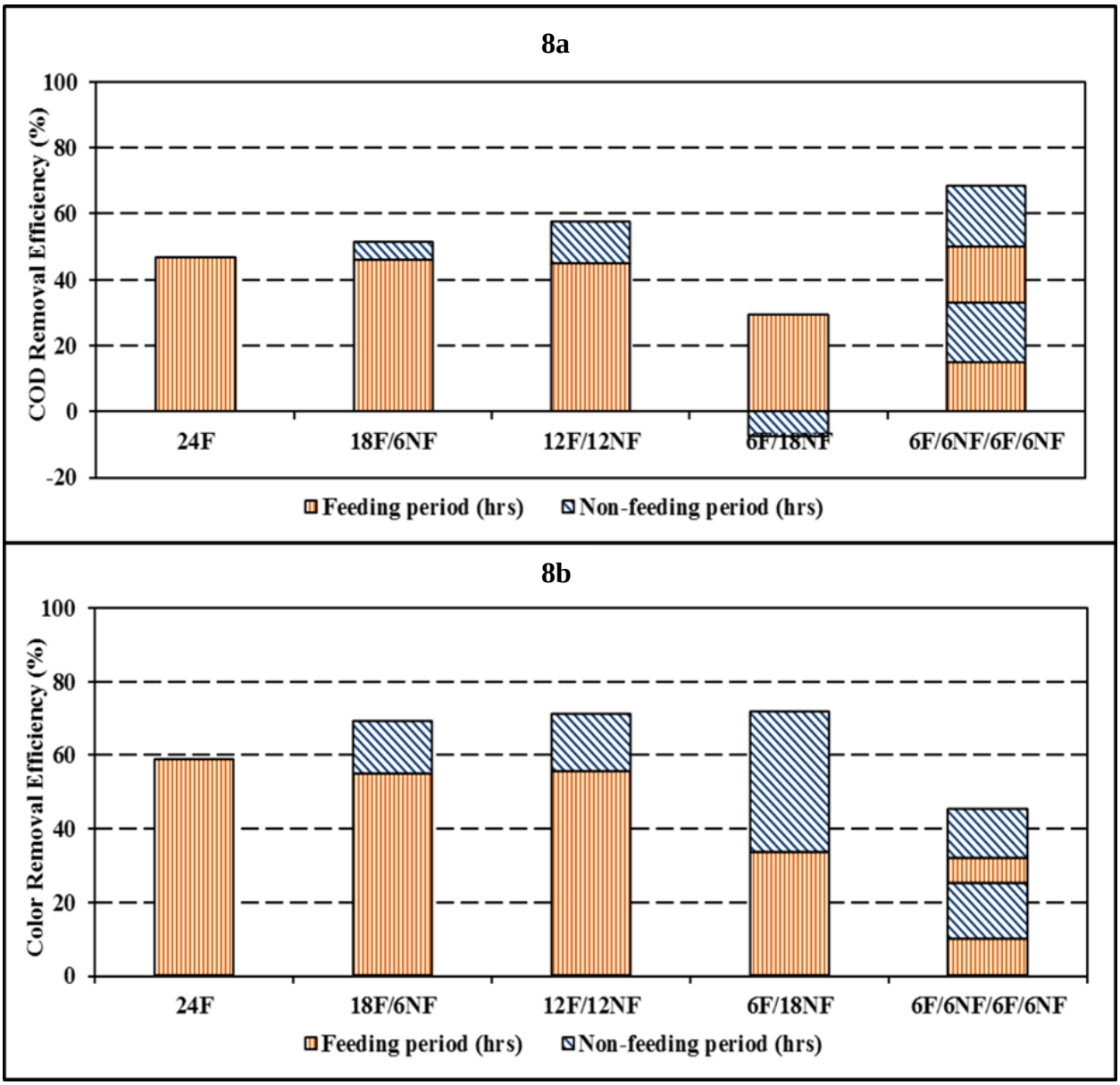


Figure 8: Removal efficiencies at continuous/intermittent combinations (8a) COD removal efficiency (8b) Color removal efficiency

The intermittent period was introduced for the fact that dyes are complex organic source for biomass and need stabilization period for complete assimilation. Shorter non-feeding period of 6 hrs was introduced to increase the biomass adaptation to complex substrate with 18 hrs continuous feeding at 2.67 kg COD/m^3-d of OLR and 24 hrs HRT (Run 2). The increased OLR of 2.67 kg COD/m^3-d during continuous period is the extra amount of food provided to biomass during non-feeding period. The COD and color removal rates were measured before and after the non-feeding period to analyze the effect of provision of stabilization phase on substrate biodegradation. The COD removal analyzed after non-feeding (intermittent) period gave tCOD of the whole run. The analysis showed a positive effect of intermittent phase with increase in COD removal from 45.92 to 51.50% and color removal from 54.80 to 69.04%. During feeding period, not all the substrate was bio-assimilated and mostly got desorbed due to overloading the adsorption capacity of biomass. This verified the theory that intermittent period forces the microbes to feed on the complex substrate when easily degradable glucose is not available anymore. Since dyes act as a complex organic source, the feeding on dyes leaded to their assimilation into harmless end products. It is to be noted here that non-feeding period of run contributed less in tCOD removal as compared to continuous period. This indicates that main substrate removal mechanism is adsorption (physical entrapment) followed by microbial degradation of adsorbed substrate during non-feeding period which leads to complete substrate removal (Coelho et al., 2007; Nadais et al., 2005).

Nadias et al., (2005) found that shorter feedless period is not sufficient for complete biodegradation of accumulated complexes on sludge bed. Thus, the non-feeding period was extended to achieve better assimilation of substrate. This was done by providing 12 hrs continuous feeding followed by 12 hrs non-feeding period. A load of 4 kg COD/m^3-d OLR was

applied during continuous operation of reactor at 24 hrs HRT (Run 3). This loading didn't exceed the adsorption capacity of substrate on biomass, leading to better assimilation of complex substrate and increasing the COD and color removal rates to 57.50% and 71.04% respectively. Coelho et al., (2007) also stated its findings that UASB 2 reactor with longer feedless period of 9 hrs with double substrate concentration provided better methanization rate as compared to UASB 1 reactor with shorter feedless period. UASB reactor underwent problems like reactor leakage, power failure and faulty heating equipment during Run 3. These problems were reflected in the effluent COD and dye residual concentrations. The reactor was preceded with same operating conditions and the run was operated till steady state is achieved.

Since extension of non-feeding (intermittent) period showed marked positive response on complex substrate removal rates, the 12 hrs non-feeding period was extended to 18 hrs at increased OLR of 8 kg COD/m^3-d during continuous feeding for 24 hrs HRT (Run 4). Though UASB reactor was reported to resist high organic loading rates, sudden increase in OLR from 4 (Run 3) to 8 kg COD/m^3-d (Run 4) reduced the COD removal efficiency to 29.40%. Further 18 hrs non-feeding period forced the microbes to starve for food for long and put the biomass into endogenous phase. Productions of SMPs (soluble endogenous products) by bacteria were reflected in effluent COD concentration. Though increased color removal of 71.82% was observed for 18 hrs non-feeding phase, color was only removed by adsorption phenomena and not all dye particles adsorbed were fully assimilated. Much of dye's colorless intermediate metabolites contributed in increased COD concentration, decreasing tCOD removal to 21.80%. Similarly, this also indicated that effluent COD concentration obtained after short feeding period is always higher than obtained after longer feed periods. For Run 3, the average effluent COD concentration was 1055.50 mg/L after 12 hrs continuous feeding which was reduced to 908.91

mg/L after stabilization period of 12 hrs. However, for Run 4, the average effluent COD concentration was 1465.90 mg/L after 6 hrs feeding period which further increased to 2175.40 mg/L. This means that continuous high load for shorter HRT leads to exhaustion of sludge retention capacity resulting in drop in reactor performance as well.

Variation in COD removal rate for each day was observed throughout the experimental Run 4. This was due to uneven assimilation of dyes in addition of glucose as substrate that leads to higher COD removal rates for some days while sometimes dye residuals in effluent increase the effluent COD concentration. In order to avoid the error due to variations occurred; adequate amount of substrate availability with stabilization period may improve reactor performance. Thus, a combination of 6F/6NF/6F/6NF was operated at 4 kg COD/m^3-d OLR during feeding periods for 24 hrs HRT (Run 5). This was equally extended at 6 hrs feeding with 6 hrs non-feeding periods. The maximum COD removal of 68.5% was obtained with lowest color removal rate of 45.14%. Low color removal was due to excessive overloading of biomass leading to desorption of already accumulated dye concentration with equally less stabilization period for complex dyes. Glucose was readily available to biomass and was assimilated by microbes during short non-feeding period giving increased COD removal efficiency. However, lower COD removal was observed during continuous feeding as compared to intermittent feeding. This was due to improved substrate (glucose) degradation during non-feeding period. This observation was similar to Couras et al., (2014) who observed the effect of fat shock by raising the feed fat from 110 to 261 mg/L in intermittent systems and 63 to 130 mg/L in continuous systems. Intermittent systems showed no significant effluent COD or TSS removal efficiency however, COD removal rate was reduced during continuous system.

4.5.2 UV-Vis Spectrum Scan Analysis

The color removal rates can be further observed by significant changes in peaks in UV-Vis spectrum of influent and effluent samples during single dye feed studies. Figure 9 showed that structural changes due to biodegradation of single influent dye used during 12F/12NF (Run 3). The influent spectrum had a visible peak at 488 nm in visible region (350 – 1100 nm) while effluent showed the absence of peak where the curve linearly dropped. The disappearance of absorbance peak at 488nm reflected as evidence of decolorization and breakdown of chromophoric group of Bezektiv Cosmos Orange dye. The linear curve started at higher absorbance giving indication of peak below 350 nm in UV region. The presence of peak at lower wavelength showed the occurrence of colorless aromatic amines which are not properly assimilated (Işik & Sponza, 2008; Somasiri et al., 2006). This showed that biodegradation was mainly due to adsorption of dyes on biomass and not due to microbial assimilation. The biodegradation pathway proposed by (Manu & Chaudhari, 2002) and (Ong et al., 2005b) under anaerobic conditions mentioned that Orange Ⅱ upon reduction produces 1-amino-2-napthol and sulfanilic acid. The FTIR spectrum (not shown) of dried Bezektiv Cosmos Orange dye powder also confirmed the presence of sulphonic group which upon biodegradation must have generated the intermediate sulfanilic acid observed at 329 nm. The minimal traces upon degradation can also be detected by spectrum obtained from effluent sample.

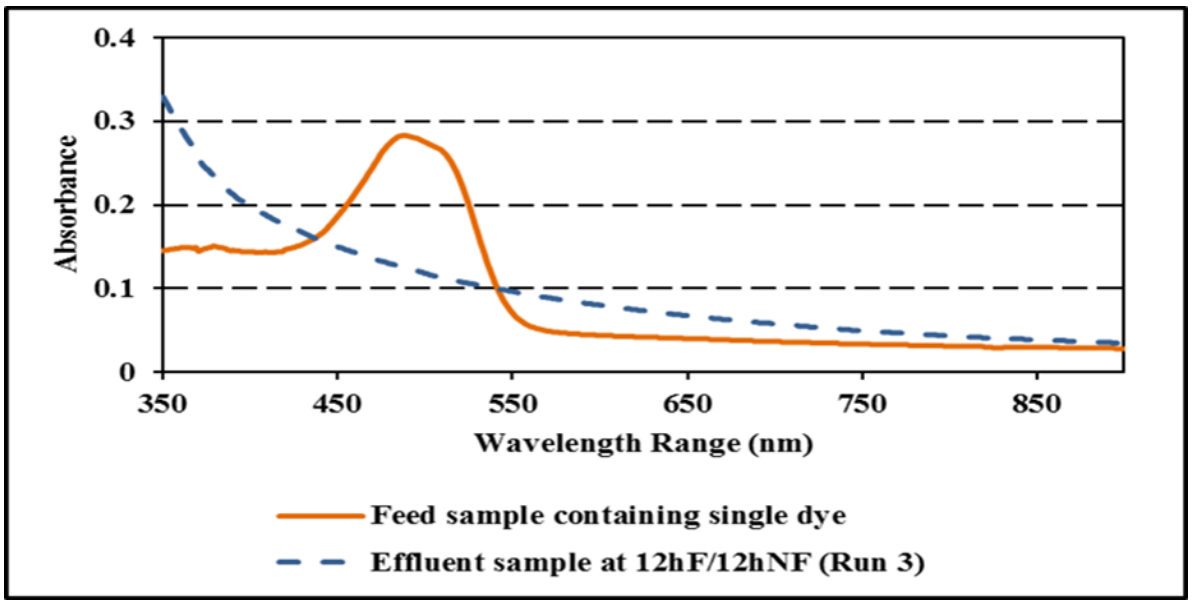


Figure 9: UV-Vis spectrum of single dye used in influent and effluent

4.5.3 Reactor Stability Parameters

Effluent sample was analyzed for VSS/TSS, VFA/alkalinity and pH which depicts the stable operating conditions of the whole reactor (Figure 10). At the beginning of Run 1, heavy flotation of biomass was observed at top section of reactor due to continuous upflow regime of reactor and presence of non-settled granules. Thus, the VSS/TSS ratio exceeded the desired range to 0.67. However, with adaptation of sludge to substrate nature and improved retention of acclimatized granules, non-feeding period provided diminishes the biomass washout. During 18F/6NF (Run 2), with small retention period given, the ratio was stabilized between 0.4 - 0.6 which was maintained for the operational cycle of 12F/12NF (Run 3) as suitable non-feeding period reduces the biomass flotation and washout. Due to increased continuous feeding for small HRT of 6 hrs during 6F/18NF (Run 4), a slight increase in VSS concentration was found in effluent due to increased inflow velocity as they drag the fine solids to outflow. But the overall VSS/TSS ratio was well within range of 0.56 for extended 18 hrs non-feeding periods. With effluent

recirculation, the effect of decrease in HRT was clear as it was dependent on inflow/recirculation ratio. However, this ratio was disturbed again to 0.63 when consecutive feeding of 6 hrs was done after small non-feeding period of 6 hrs (Run 5).

Another stability parameter to optimize the anaerobic treatment is pH which must be in range of 6.6 -8.2 (Latif et al., 2011; Somasiri et al., 2006). Due to presence of dyes toxicity in feed, an acidogenic and facultative methanogenic culture was found in microbial consortia which if buffered properly, will help to achieve better color removal rates. Here, not all the H_2 and acetic acid formed by acidogenic and acetogenic bacteria were converted to methane. Due to higher concentration of VFAs in reactor, carbonate alkalinity generated by biomass was consumed and pH was not in the desired range. To maintain the pH in reactor, external bicarbonate alkalinity in form of sodium bicarbonate was added as per requirement to get the pH in desired range. pH of 6.75 was found during continuous feeding at 24 hrs HRT. With extension of non-feeding periods from Run 2 to Run 3 and incapability of biomass to generate enough carbonate alkalinity, a drop in pH till 6.10 was observed due to VFAs accumulation in reactor. This drop in pH can be related with corresponding decrease in COD and color removal rates and high VSS/TSS ratio due to sloughing of biomass. External addition of 0.5g of sodium bicarbonate improved the reactor performance while maintaining the pH in the desired range. pH was largely dropped during 6F/18NF (Run 4) due to occurrence of endogenous phase for biomass, low microbial activity and buildup of VFAs in sludge, leading to addition of external alkalinity twice a day.

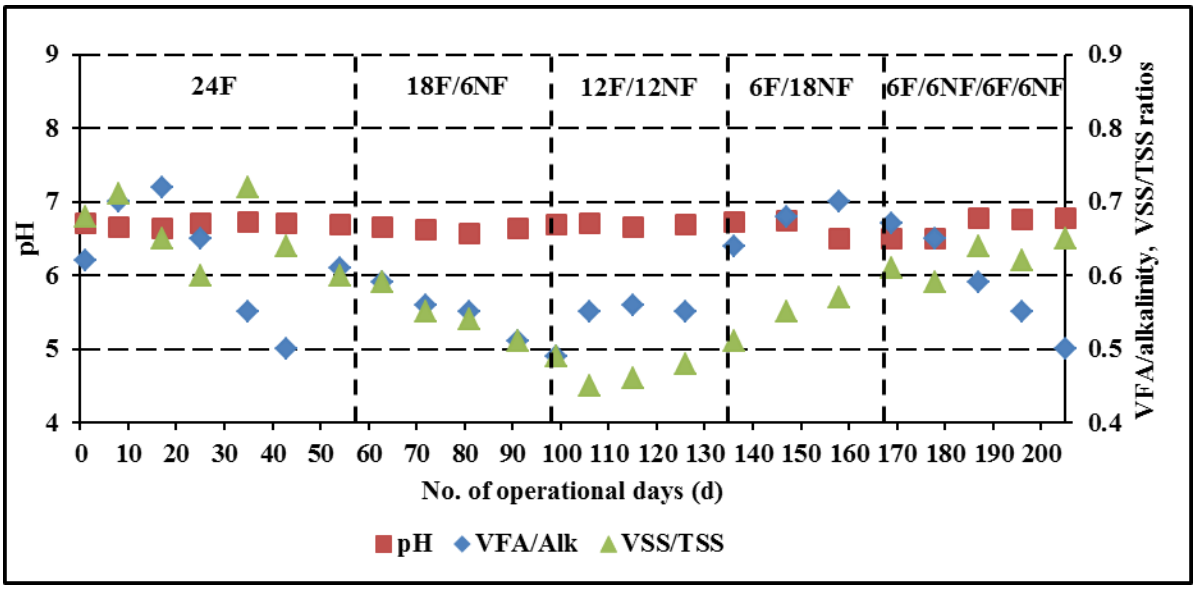


Figure 10: Reactor stability parameters under single dye feed conditions

COD removal efficiency decreases as alkalinity concentration decreases from 250 mg/L. It was reported in literature that 0.68 VFA/alkalinity ratio is obtained when alkalinity concentration is less than 250 mg/L which indicate the system unstability. The intermittent period is suitable for forced assimilation of VFA concentration found in reactor if sufficient alkalinity of 1000 – 5000 mg/L is available to biomass. The system alkalinity was maintained by recirculation of treated effluent (Latif et al., 2011). However, during experimentation the reduction in VFA concentration was gradually achieved due to conversion of acidogenic consortia into facultative methanogenic culture. Introduction of dyed wastewater in anaerobic biomass led to accumulation of VFAs up to 1052 mg/L with average VFA/alkalinity ratio of 0.65. This ratio was decreased to 0.60 at VFA concentration of 578 mg/L but reduced alkalinity generation of 963 mg/L during 18F/6NF (Run 2). 12F/12NF (Run 3) further stabilized the reactor by reducing the ratio to 0.55 at 525 mg/L of VFAs and 954.50 mg/L of alkalinity concentration. Longer non-feeding period of 18hrs during 6F/18NF (Run 4) leaded to acidic conditions in reactor with high accumulation of VFAs up to 1233 mg/L concentration at high VFA/alkalinity ratio of 0.71. 6F/6NF/6F/6NF gave

optimum VFA/alkalinity ratio of 0.52 at VFA concentration of 425 mg/L which is the minimum concentration of VFAs recorded.

4.5.4 Biomass Concentration

Figure 11 showed that MLVSS/MLSS ratio of initial sludge was 0.43 at MLSS concentration of 4400 mg/L when operated for continuous operation at 24 hrs HRT (Run 1). The actual growth was observed at the end of 18F/6NF (Run 2) and start of 12F/12NF (Run 3) to 7300 mg/L at MLVSS/MLSS ratio of 0.73. The increase in MLVSS/MLSS ratio indicates higher concentration of biomass. It was reported in Nadais et al., (2008) that biomass developed under intermittent conditions has better degradation capacity of complex substrate. The drop in MLVSS (microbial concentration) to 6900 mg/L was observed during 6F/18NF (Run 4) with increased VSS concentration of 183 mg/L at VSS/TSS ratio of 0.56. This indicated the washout of inactive microbes during endogenous phase occurred due to prolonged non-feeding period.

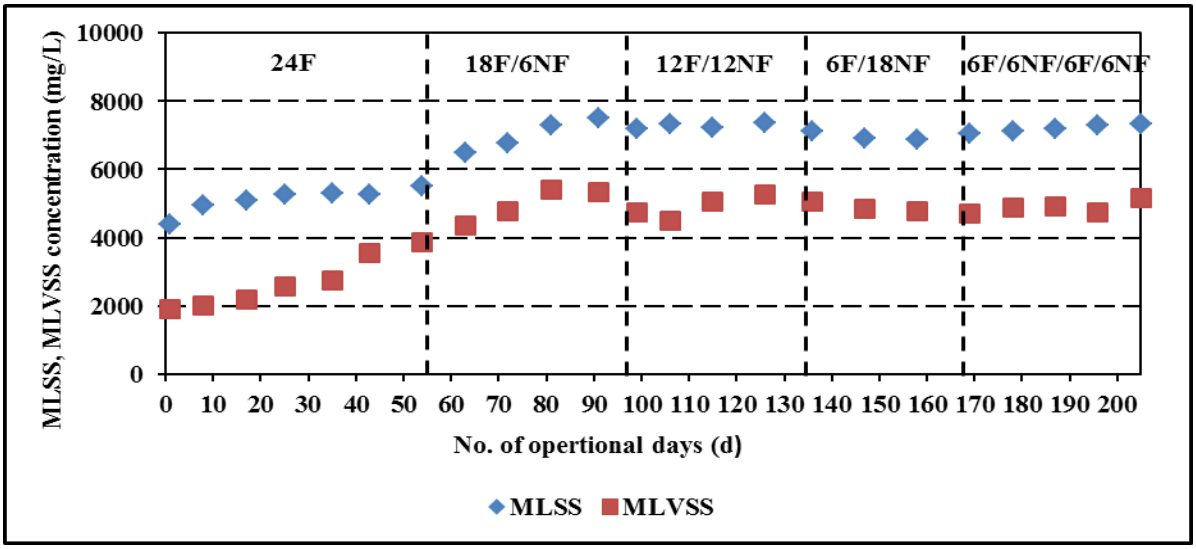


Figure 11: Biomass profile under single dye feed conditions

4.6 Mixed Dyes Feed at Optimized Intermittent Conditions

4.6.1 Mixed Dyes Removal Rates

The optimum operating condition of 18F/6NF was examined for treatment of mixed dyes simulated textile wastewater feed containing three dyes of different classes (reactive, disperse and vat) at 10 mg/L concentration respectively. This gives the increase in total dye concentration to 30 mg/L at 24 hrs HRT and 2.67 kg COD/m^3-d OLR during the feeding period of cycle (Run 6). The reactor showed stress in its performance due to increase in dyes concentration. Bezektiv Orange (reactive dye) gave reasonable removal of 32.50% at 6.74 mg/L effluent residual concentration as biomass was already acclimated to the dye's chemical nature (Figure 12). Novasol Navy (vat dye) gave highest removal rate of 49% at 5.19 mg/L residual concentration indicating that it was readily adsorbed on biomass at short retention period of 6 hrs. Among three dyes, Foron Red (disperse dye) gave the lowest removal rate of -28% (12.82 mg/L). This resulted in increase in the effluent COD (1250 mg/L) and residual dye concentration (24.70 mg/L), decreasing the overall tCOD and color removal efficiency to 37.50% and 17.60% respectively. Senthilkumar et al., (2011) reported that increase in dye concentration increases the pH but lowers the COD removal efficiency and affects the dye metabolic rate. The pH of reactor was in stabilized range but COD and color removal decreases due to intractable nature of Foron Red dye. The dye covered the granule surface, reducing its biodegradability efficiency and desorbed at overloading point. This may also be due to smaller size of granule particles acclimatized at anoxic conditions and less availability of adsorption space for substrate. Since it was not completely assimilated into colorless amines, it contributed in COD and color removal rates.

The operating condition was shifted to 12F/12NF with increase in OLR to 4 kg COD/m^3-d during continuous feeding period of cycle at 24 hrs HRT (Run 7). Bezektiv Orange and Novasol Navy gave further reduced effluent residual concentration to 4.79 and 2.21 mg/L giving 52 and 77.80% removals respectively in total color removal rate (Figure 12). Foron Red was retained on granule surface where its effluent residual concentration was reduced to 11.55 mg/L. This showed the desorption of dye in effluent but in lesser concentration as compared to previous run giving improved color removal efficiency of -15.50%. The color removal was increased to 38.35% (18.50 mg/L) but COD removal was slightly enhanced to 47.80% (1044 mg/L). Thus, increase in non-feeding period helped in recovery from the overloading faced during previous run. Increase in OLR from 2.67 to 4 kg COD/m^3-d indicated that adequate substrate is required as electron donor for corresponding color removals. The overall decrease in COD and color removal rates were observed as mixed dyes who didn't behave as suitable electron acceptors at 24 hrs HRT. Isik & Sponza, (2008) reported 77% of COD and 67.2% of color removal in UASB reactor treating Procion Red H-37B at 3.1 kg COD/m^3-d OLR and 24 hrs HRT and stated the removal efficiency to be insufficient. Thus, to obtain further efficiency, increase in HRT was required.

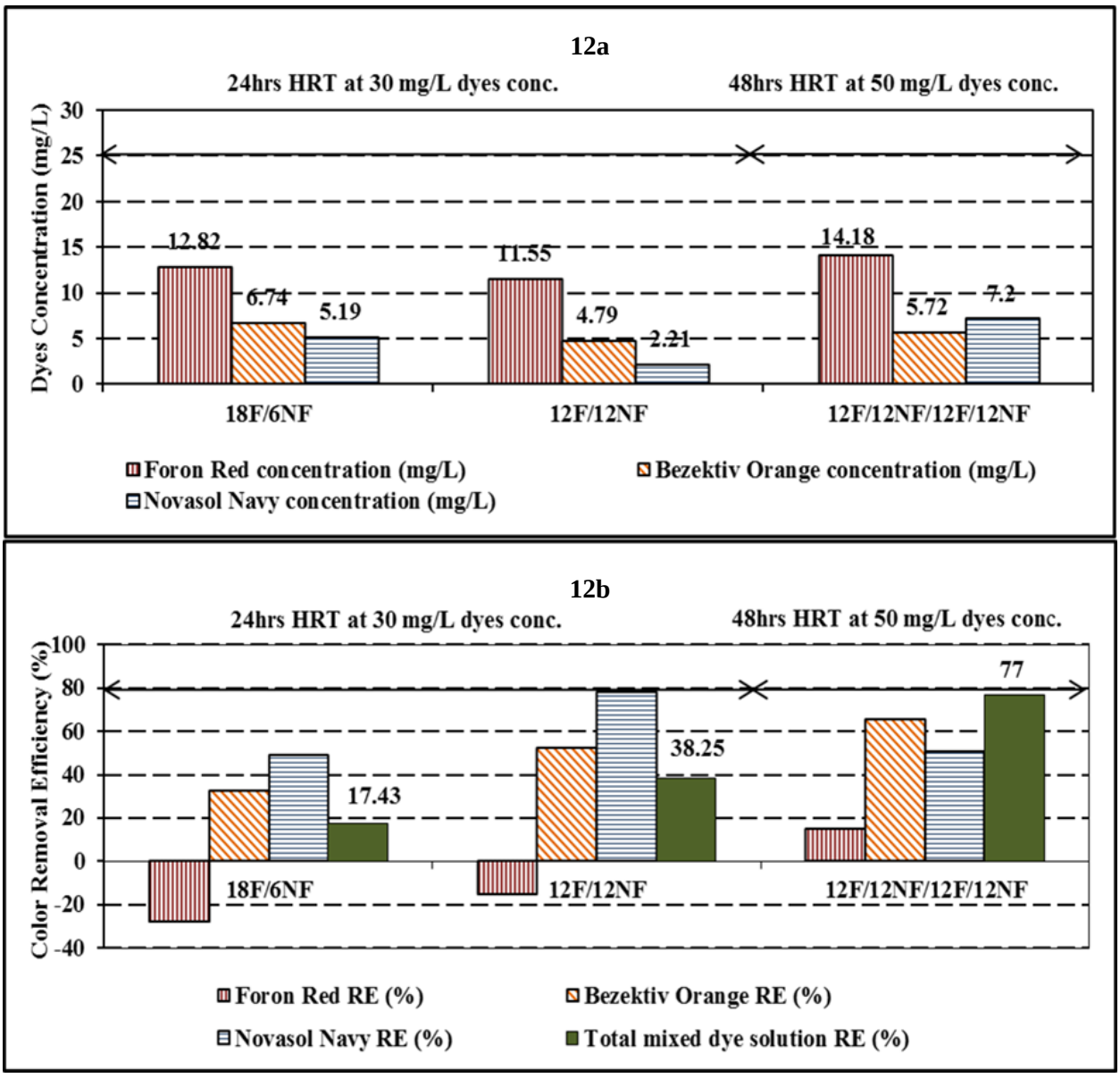


Figure 12: Dyes removal rate at optimized intermittent combinations (12a) Effluent dyes residual concentration (12b) Color removal efficiency

Increase in dyes concentration from 30 – 50 mg/L gave an overall increase in residual dye concentration in effluent. This also resulted in high decolorization rates at 2 kg COD/m^3-d of OLR when cycle was extended to 48 hrs HRT with 12F/12NF/12F/12NF operating run (Run 8). Somasiri et al., (2006) reported the residual dye concentration of 0.4 mg/L at 10 mg/L influent dye concentration while 18 mg/L persisted at 300 mg/L dye concentration. This gave an increase

in color removal efficiency to 71% but increased residual dye concentration of 14.12 mg/L in effluent. To avoid the endogenous phase at same OLR of 2 kg COD/m^3-d, consecutive feeding and non-feeding periods for 12 hrs were operated. This helped in assimilation of more substrate to end- products. Bezektiv Cosmos Orange and Novasol Navy removal efficiency was improved to 65.50 and 56.58% respectively. Foron Red dye gave 14.50% removal efficiency at residual concentration of 14.18 mg/L. Lourenco et al., (2001) reported the effect of operational parameters on dyes biodegradation in SBR with increase in Remazol Brilliant Violet 5R dye concentration from 60 -100 g/L at SRT of 10 -15 days and described that bacteria in anaerobic/aerobic biomass bring exhaustion of carbon source in feed early, favoring expansion of dye metabolizing microbial consortium. Longer HRT of 48 hrs enabled the granules to adsorb the sudden increase in dye concentration at low inflow velocity. Ong et al., (2005b) also reported the COD and Orange Ⅱ removal efficiencies to be increased from 27 to 35% and 82 to 97% by increasing the HRT from 24 to 48 hrs and dye loading rate from 0.06 to 0.30 g COD/L-d at 30^{o}C in lab scale UASB-SBR system

4.6.2 UV-Vis Spectrum Scan for Mixed Dyes Feed

The effluent samples from Run 7 and 8 were again analyzed for spectrum obtained from UV-Vis spectrophotometer in visible range (350 – 1100 nm) as shown in Figure 13. This was done to analyze the degradation pathway adapted by mixed dyes feed solution. The influent curve from Run 7 (30 mg/L at 24 hrs HRT) showed two peaks at 401 nm and 577 nm in visible range. Similar peaks were observed for influent sample from Run 8 (50 mg/L at 48 hrs HRT) but at lower absorbance values. The effluent curve for Run 7 show the linear curve with disappearance of correspondence influent peaks showing the rupture of chromophoric structure of dye and

production of small amount of colorless dye metabolites or residuals near UV range at 371 nm. With increase in HRT to 48 hrs for Run 8, the effluent peak starts at the same absorbance point as influent peak showing the possibility of absence of any aromatic amines and their assimilation into end products. One peak at 589 nm showed the possible dye residuals obtained after Foron Red reduction (528 nm) while other curves indicated dye residuals in effluent at 488 nm.

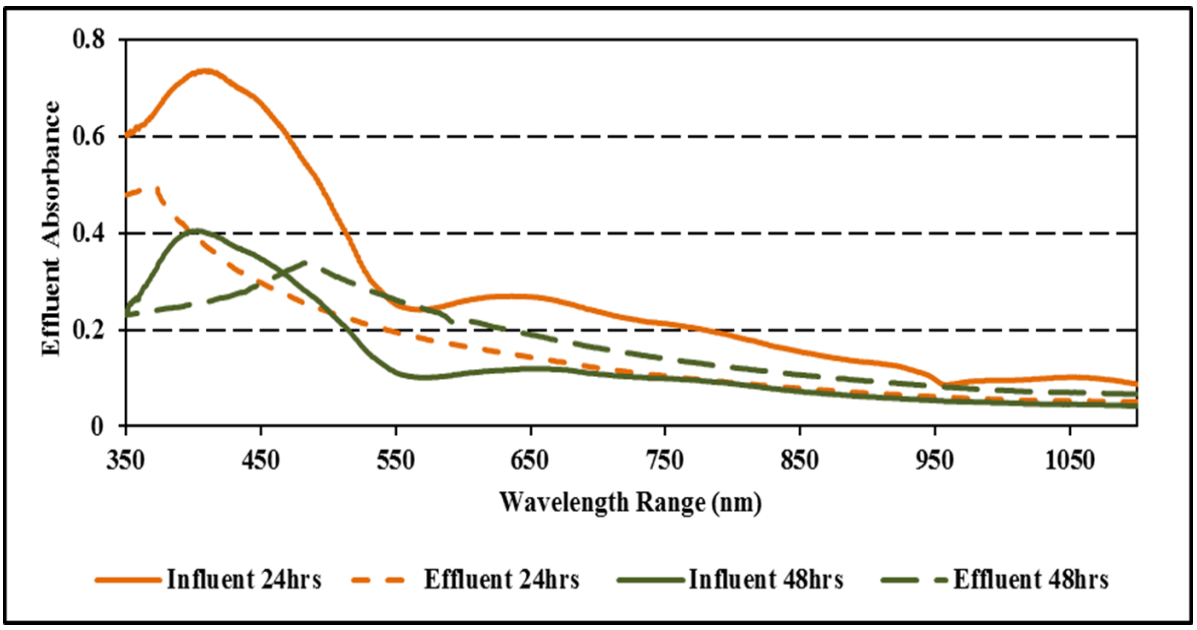


Figure 13: UV-Vis spectrum of mixed dyes in influent and effluent

4.6.3 Biomass Adaptation to Mixed Dyes Feed

A drop in MLSS from 6780 to 5900 mg/L was observed due to increase in dye concentration as a shock. A quick recovery was observed when non-feeding period was extended to 12 hrs and better sludge acclimatization to substrate was achieved. Here, MLSS was maintained at 6300 mg/L (Figure 14). A better MLVSS/MLSS ratio of 0.85 was observed with high biomass concentration when HRT was extended from 24 to 48 hrs. This was accompanied by stable pH between 6.70 - 6.98 and VFA/alkalinity ratio of 0.49 at 370 mg/L VFA and 755 mg/L alkalinity concentration (Figure 15).

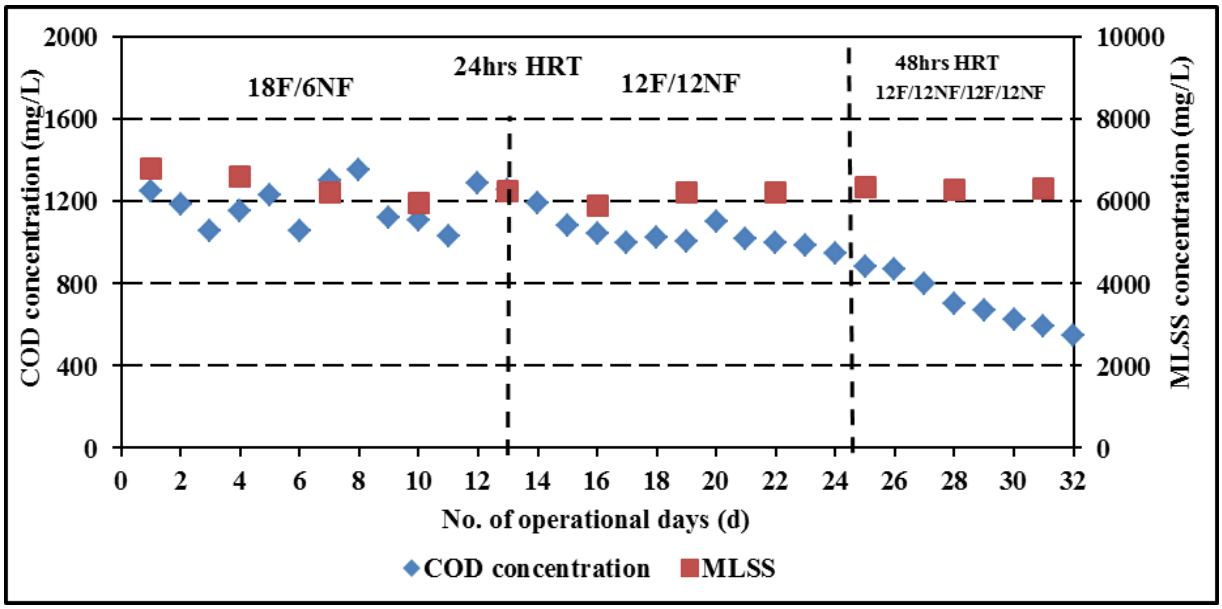


Figure 14: Biomass profile at optimized intermittent combinations under mixed dyes feed conditions

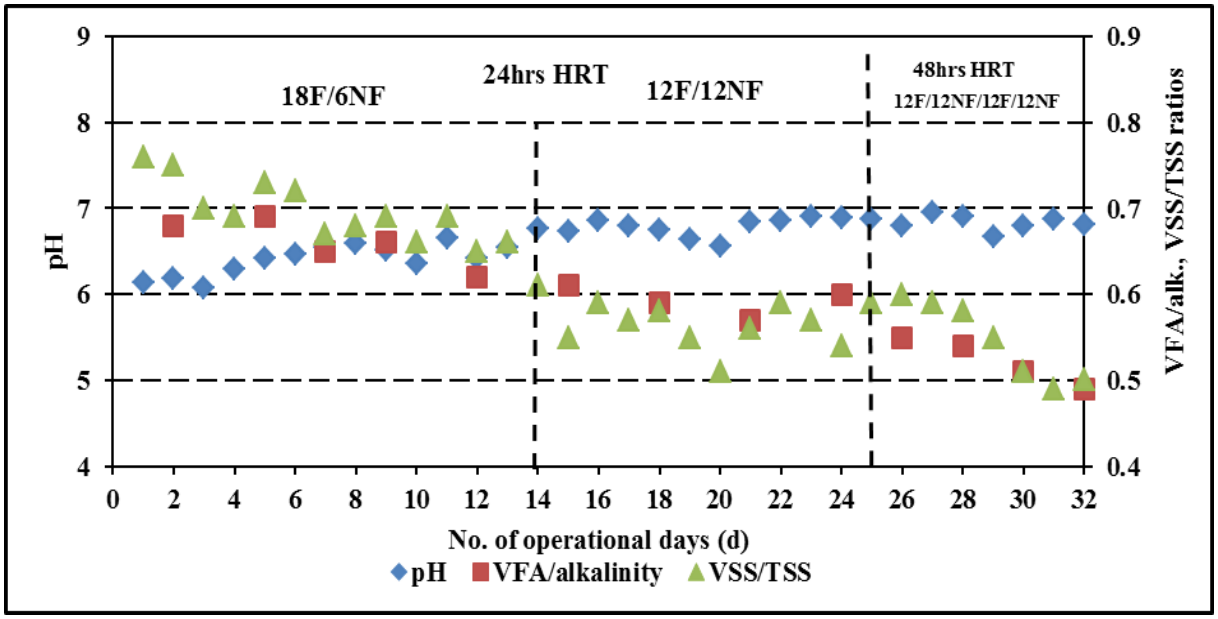


Figure 15: Reactor stability parameters at optimized intermittent combinations under mixed dyes feed conditions

4.6.4 Total Kjeldahl Nitrogen (TKN) and orthophosphates (PO_4^{-3}-P) Removal Rates

Total Kjeldahl Nitrogen (TKN) and orthophosphates (PO_4^{-3}-P) concentration was analyzed in effluent sample for last three runs operated for mixed dyes simulated textile wastewater treatment studies. Asadi et al., (2012) reported an increase in nitrogen and phosphorus removal rates with decrease in HRT and increase in OLR. A contradictory observation was seen during the experimental runs. Initially, an increase in TKN concentration in effluent was observed for 18F/6NF (Run 6) which continued to rise linearly to 12F/12NF (Run 7) with increase in concentration to 56 mg/L and giving 72% removal rate (Figure 16). Nitrogen removal is achieved by aerobic nitrification and anoxic denitrification using autotrophic nitrifying and heterotrophic denitrifying bacteria. But here, reduced TKN removal rates were observed at extended non-feeding period of 12 hrs due to assimilation of complex substrates (glucose and dyes). Due to insufficient organic source available and restriction in anoxic conditions, accumulation of TKN was observed in effluent. It is reported that at increased HRT, nitrification rate is higher than denitrification rate resulting in reduced removal rates (Lourenço et al., 2001). However, contrary to this, at increased HRT of 48hrs, consecutive feeding and non-feeding periods promote the nutrient uptake requirement for efficient biomass growth. This led to reduced TKN effluent concentration to 42.35 mg/L with 78% removal efficiency. Ozgun et al., (2015) using AnMBR treatment, having UASB coupled with ultrafiltration membrane, reported the inability of removal of carbohydrates and proteins from UASB effluent. Asadi et al., (2012) described in his study that due to increase in HRT, TN removal efficiency was reduced due to insufficient carbon source and restriction in anoxic conditions. The maximum TN removal achieved was 59.9% at 12 hrs HRT and this decreased at longer anaerobic HRTs due to higher nitrification rate as compared to denitrification rate.

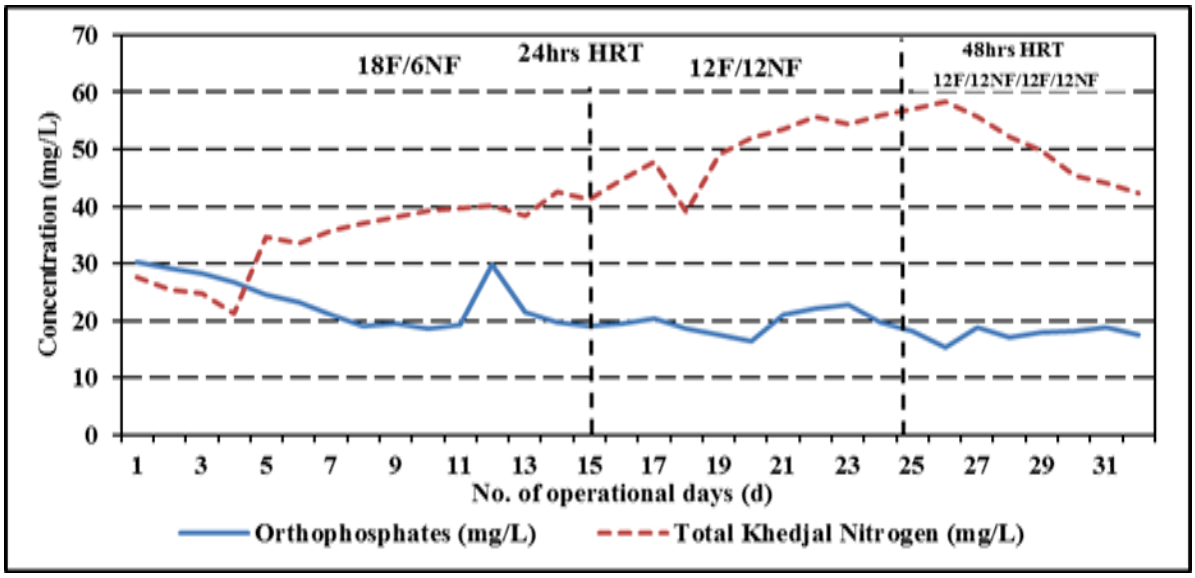


Figure 16: Nutrient removal rate at optimized intermittent combinations under mixed dyes feed conditions

UASB reactor showed no significant phosphates removal efficiency due to its limited capacity to support biomass growth. Due to minute biomass growth with no SRT, nutrient demand for microbes was negligible. A little accumulation of phosphates was observed during 18F/6NF (Run 6) with no further accumulation observed at 17 -18 mg/L effluent concentration. Thus, it created a balance in the system with total phosphates concentration in and out of reactor to be same (Ndegwa, 2008). The slight removal efficiency is the removal of particulate nutrients by sedimentation and filtration and therefore, showed little removal efficiency. Asadi et al., (2012) reported that absence of aerobic reaction phase and increase in HRT leads to inhibition of phosphorus accumulating organisms (PAOs).

5 CONCLUSION AND RECOMMENDATION

5.5 Conclusion

In this study, the effect of continuous and intermittent operation in single anaerobic UASB reactor was observed on the treatment of simulated textile wastewater. The efficient biodegradation of glucose and dyes were detected with varied feeding and non-feeding periods at 24hrs and 48hrs HRT and their impact on reactor stability parameters were observed. Important findings are as under

- Extension of HRT from 24 to 48 hrs with consecutive feeding and non-feeding periods of 12 hrs (12F/12NF/12F/12NF) in single anaerobic UASB reactor lead to better assimilation of adsorbed COD (glucose) and dyes intermediate metabolites in simulated textile wastewater.
- Parameters indicating stability of reactor i.e. pH and VFA/alkalinity ratio were maintained by daily addition of 0.5 g of sodium bicarbonate, whose frequency decreased with increase in HRT from 24 to 48 hrs.
- UV- Vis spectrum scan showed the possible assimilation of dye intermediate metabolites at 48 hrs HRT produced during 24 hrs HRT.
- Insignificant TKN and Phosphates-P removal efficiency was observed indicating the accumulation of nutrients in sludge bed of reactor.

5.6 Recommendations

- Improvement in anaerobic sludge from flocculent to granulated state will improve the substrate removal proficiency of reactor.
- Close monitoring of reactor stability parameters by bringing the pH to neutral and VFA/alkalinity ratio below 0.4
- Detailed granulated sludge examination treating real textile wastewater and using intermittent operation of reactor.
- Single anaerobic treatment of dyed textile effluents is insufficient and requires aerobic post treatment for complete decolorization and degradation of organics.

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