Biofouling Of High Purity Water Systems

By

Amani Fathei Shaeikh

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Under The Supervision Of
Dr. Brian Corcoran

School Of Mechanical And Manufacturing Engineering
Dublin City University
Ireland

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Preface

Declaration

I hereby certify that the material, which I now submit for assessment on the programme of study leading to the award of Degree of Master of Engineering is entirely my own work and has not been taken from the work of others save and to the extent that such work has been cited and acknowledged within the text of my work.

Signed: .....

ID No : 51184001

Date : 22 Sept 2004

Acknowledgements

I give this thesis to my parents who tried to compensate me for ten years I lost by injustice…

My deep thanks and appreciation to Professor SALEM HASHMI and Dr. BRAIN CORCORAN who give me this great chance….

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ABSTRACT

Biofouling Of High Purity Water Systems

The fouling of high purity water can be quantified by comparing the effect of fluid velocity (laminar and turbulent) on the accumulation of cell on tube surfaces. Direct observations were monitored under light microscope to quantify numbers of the cells sedimented on a of glass slide inserted into the tube. CFD (Computational Fluid Dynamics) software, fluent was used to analyse the problem. Results show the increase of cell count coincided with the increase of the water velocity resulted in increase in the fluid fractional resistance.

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CFU	**Cell For Unit**
EU	**Europium**
DOC	**Dissolved Organic Carbon**
TOC	**Total Organic Carbon**
DOC	**Dissolved Organic Carbon**
DO	**Dissolved Oxygen**
Na	**Sodium**
Si	**Silicon**
Ca^{++}	**Calcium ions**
Mg^{++}	**Magnesium ions**
Fe^{++}	**Iron ions**
Ni^{++}	**Nickle ions**
Cu^{++}	**Copper ions**
H^{+}	**Hydrogen ions**
OH^{-}	**Hydroxyls**
CL	**Chloride**
Pb	**Lead**
SO_4	**Sulphate**
H_2O_2	**Hydrogen peroxide**
$CaCO_3$	**Calcium carbonate**
$CaSO_4$	**Calcium sulphate**
H_2S	**Hydrogen sulfide**
CO_2	**Carbon Dioxide**
NH_3	**Ammonia**
UF	**Ultra filtration**
RO	**Reverse osmosis**
ULPRO	**Ultra-Low Pressure Reverse Osmosis**
DI	**Deionisation**
HD	**Holding Distribution**
PVC	**Poly-Vinyl-Chloride**

PP	**Poly-Propylene**
PVDF	**Poly-Vinylidene-Fluoride**
PEEK	**Poly-Ether-Ether-Ketone**
UV	**Ultra Violet**
CBL	**Concentration Boundary Layer**
HBL	**Hydrodynamic Boundary Layer**
CFD	**Computational Fluid Dynamics**
FM20	**Centrifugal Pump Demonstration Unit**

CHAPTER 1

LITERATURE REVIEW

1.1 High Purity Water (HPW)

Purified Water is water obtained by distillation, ion-exchange treatment, reverse osmosis, or other suitable process. It is prepared from water complying with the regulations of the U.S. Environmental Protection Agency (EPA) with respect to drinking water. It contains no added substances. High purity water is unique in that it is the only component, which must be produced by the manufacturer, because it is not available from a vendor in a ready-to-use form. Water is utilized in the production of every type of pharmaceutical; in some products, such as parenterals, it is a critical component [72].

High purity water (Figure 1.1), particularly because it has been freed of ions, is very corrosive. It is described as "hungry water," and is highly corrosivity. Deionized water display a natural pH (7.0) only at 25 °C. At elevated temperature, the pH is decreasing below 7.0. The water is thus more avid in its acidic attack on metals at higher temperature because of the presence of greater concentrations of hydrogen ions [8].

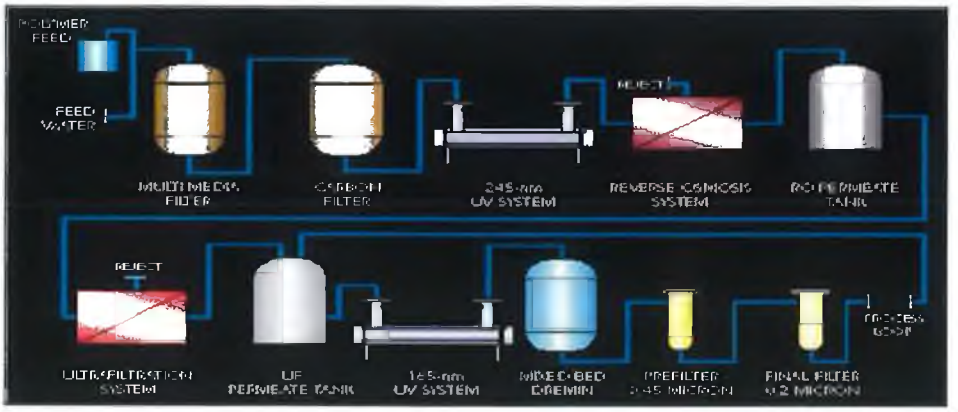


Figure 1.1. Schematic diagram of the simplified HPW system

The high purity water is expected to be essentially sterile and have a low level of endotoxine and have a low number of micro-particles of organic or inorganic origin, DOC (Dissolved Organic Carbon). So-called high purity water must have a resistivity of greater than 0.1 MΩ.cm (less than 5.0 ppm). It should be noted that many specific users have stated requirements up to 18 MΩ.cm (28ppm); a variation factor of 180. The normal water or normally treated water, including potable water, is defined to have a resistvity of less than 0.01 MΩ (more than 50 ppm), Table 1.1.

Type of water	MΩ.cm	ppm
Normal	< 0.01	> 50
HPW	> 0.1	< 5.0

Table 1.1. Water Resistivity

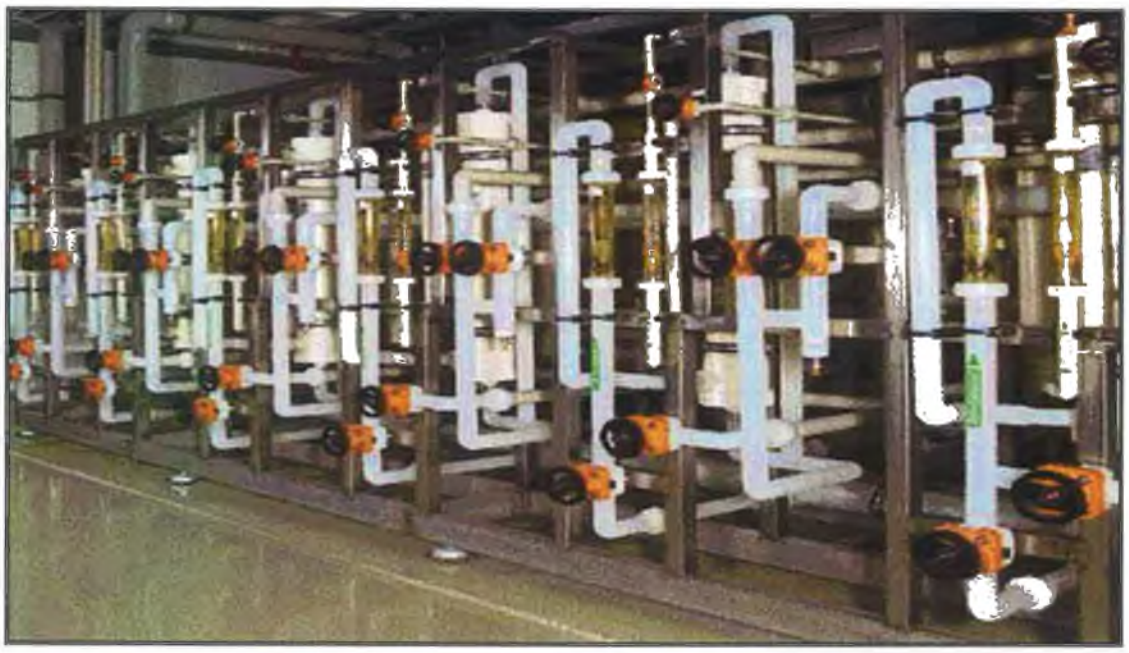


Figure 1.2. High purity water treatment plant in the electronics industry

A substance is termed "toxic" if it has a negative effect on the health of an organism. The following is a short list of toxic contaminates reported found in the water supplies:

Heavy metals	**Chlorine**	**Trihalmethanes**	**Nitrates**
Bromoform	**Dimethylfrmamide**	**Coliform Bacteria**	**Nitrites**
Nitrosomines	**Methylene chloride**	***Giaria lamblia***	**Fluorine**
Chlordane	**Chloramines**	**Pesticides**	**Sulfates**
Phthaltes	**Bromobenzene**	**Viruses**	**Arsenic**
Ozone	**Bromochloromethane**	**Acetaldehydedioxide**	**Asbestos**

Table 1.2. List of toxic contaminates in water supply

Pharmaceutical water needs to be absolutely free of these contaminates to ensure they do not reach the final products [8]. Highly purified water is utilized in the biopharmaceutical and microelectronics industries. The former uses high purity waters for production, processing, formulation, cleaning, and rinsing. Biopharm operators are primarily concerned with microbial, chemical, and endotoxin contaminants that may compromise standards of safety, efficacy, strength, purity, and quality of a drug. There are two commonly used grades of pharmaceutical bulk water: water for injection (WFI) and purified water (WPU). In microelectronics, high purity water is typically called ultrapure water (UPW). Microchip fabricators are concerned with particulate, ionic, and organic contamination detrimental to the integrity of microchipcircuitry. The majority of UPW is used for wafer cleaning, rinsing and process equipment component cleaning.

Based on different grades of the purity required, HPW can be classified into four categories as shown in table 1.3. Classes I and II are termed as high grade HPW, which is consumed by wafer related processes such as wafer bumping, etching, backgrinding, sawing, etc. Some other indirect users such as failure analysis (FA), reliability test (RT) and quality assurance (QA) consumes very small amount of high grade HPW. Classes III and IV are termed as low grade HPW, which is consumed by IC related processes such as electroplating, singulation, marking, etc. The detailed quality specifications of Classes I–IV HPW are summarized in table 1.3, which is based on the standard HPW guidelines [73].

Parameter	UOM	Class I	Class II	Class III	Class IV
Resistivity	MΩ cm	18.0	16.0	12.0	0.5
TOC	ppm[a]	0.01	0.5	1.0	2.0
Bacteria	cfu/ml[b]	0.1	1	10	100
Silica	ppm	0.003	0.02	0.5	1.0
Ions					
Chloride	ppm	0.001	0.03	0.05	1.0
Fluoride	ppm	0.001	–	–	–
Sulfate	ppm	0.001	0.01	0.05	0.5
Ammonium	ppm	0.001	–	–	–
Copper	ppm	0.001	0.01	0.02	0.5
Potassium	ppm	0.001	0.01	0.02	0.5
Sodium	ppm	0.001	0.01	0.05	1.0

[a] ppm: parts per million. [b] cfu/ml: colony forming units per millilitre.

Table 1.3. Quality requirements of HPW at different grades of purity

1.2 The Action Limits

The quality of ultrapure water decrease due to various causes while passing through long and complicated piping. Possible causes are dissolved and/or deabsorption of impurities from the piping wall, propagation of bacteria, and penetration of air and gases into the inside of the piping [36].

Impurities in the process water such as bacteria, organics, ionic contaminations and particles are a potential cause of reduced device yield and reliability failures.
Organisms exist in a water system either as free floating or attached to the walls of the pipes and tanks. When they are attached to the walls they are known as biofilm, which continuously sloughes off organisms. Thus, contamination is not uniformly distributed in a system and a sample may not be representative of the type and level of contamination. A count of 10 CFU/ml (Colony For Unit) in one sample and 100 even 1000 CFU/ml in a subsequent sample would not be unrealistic. The purpose of establishing any action limit or level is to assure that the water system is under control.

Any action limit established will depend upon the overall water system and further processing of the finished product and its use [17].

The FDA "Guide to inspection of High Purity Water Systems" (July 1993) states the Agency policy is that less than 10 CFU/100 mL is an acceptable action limit, and for WFI an endotoxin level of less than 0.25 EU/ml [47]. The real concern in WFI is endotoxins. Because WFI can pass the LAL endotoxin test and still fail the above microbial action limit, it is important to monitor WFI systems for both endotixins and microorganisms [8]. The European Pharmacopeia (EP) adopted an endotoxin limit of 0.25 EU/mL for water (nonmandatory) used to dilute concentrated haemodialysis fluids. Voluntary standards in the USA are less stringent. The U.S. Pharmacopeia (USP) has not addressed this issue, but features an excellent chapter on waters [4]. Of interest is a draft standard from the working group on water for hemodialysis of the Association for the Advancement of Medical Instrumentation (AAMI). The AAMI limits for water table see table 1.4, are more lenient than the EP and Water for Injection, USP, which is also 0.25 EU/mL. Four dialysis-related monographs (endotoxin limits) are listed in the guidance section (non-mandatory) of the EP:

- Water for dilution of concentrated solutions (0.25 EU/mL),
- Solutions for haemodialysis (0.5 EU/mL),
- Solutions for haemofiltration and haemodiafiltration (0.25 EU/mL), and
- Solutions for peritoneal dialysis (0.5 EU/mL) [74].

Organization Bioburden	(cfu/mL)	Endotoxin (EU/mL)
European Pharmacopeia	< 100	< 0.25
EDTNA/ERCA (Proposed)	< 100	< 0.25
Japan Society for Dialysis Therapy	< 100	< 0.25
AAMI (Proposed RD62)	< 200	< 2

Table 1.4. Microbial Limits for Water for Dialysis Applications

In addition to the total microbial count action limit for purified water, the type of micro-organisms present must also be considered. The micro-organisms present should not be capable of growth in the product nor should they represent a potential health hazard when the product is used as directed. The organisms most commonly encountered are potentially pathogenic *Gram negative bacilli.* In an independent test, 6 of 14 microbiological contaminants of cosmetics found in process waters were *Pseudomonads*. The presence of *Gram negative* organisms in topical preparations poses a moderate threat to health [8].

In contrast, microelectronics specifications will generally not include an endotoxin requirement, but cover resistivity, TOC, bacteria, particles, dissolved oxygen (DO), silica, anions/cations, and metals. Gross contaminants such as resistivity, TOC, particles, DO, silica, and sodium are measured online continuously, while specific contaminants such as halogens, inorganic and organic species are measured individually offline. Specific action for ionics and metals are often driven by laboratory detection levels in the 10 to 100 parts-per-trillion (ppt) range. Some manufacturers will even drive specifications below the detection levels and require sample concentration for testing, although this is not yet common practice. Analytical instruments and procedures are not

regulated and can vary from site to site. At present, particle measurement is restricted to UPW, as per unobtrusive inline USP requirements [73].

For purified water systems, microbiological specifications are not as clear. USP XXII specifications that comply with federal Environmental Protection Agency regulations for drinking water are attempts by some to establish meaningful microbiological specifications for purified water. The CTFA (Cosmetic Toiletries and Fragrance Association) proposed a specification of not more than 500 organisms per ml. The USP 23 has an action guideline of not greater than 100 organisms per ml. Agency policy is that any action limit over 100 CFU/ml for a purified water system is unacceptable [8].

	Purified Water (topical solutions and cosmetics)	**Water For Injection**
Organics	<0.5 ppm TOC	<0.5 ppm TOC
Conductivity	<1.3 µ5/cm at 25°C in-line measurement*	<1.3 µ5/cm at 25°C in-line measurement*
Endotoxin by LAL	No specification	< 0.25 EU/mL
Bacteria	100 cfu/mL	10 cfu/100 mL

Table1.5. USP 23 Pharmaceutical Grade Water

In establishing the level of contamination allowed in a high purity water system used in the manufacture of non-sterile product requires an understanding of the use of the product, the formulation (preservative system) and manufacturing process. For example,

antiacids, which do not have an effective preservative system, require an action limit below the 100 CFU/mL maximum. [17].

Water For Injection Systems Regarding microbiological results are expected to be essentially sterile. Since sampling frequently is performed in non-sterile areas and is not truly aseptic, occasional low level counts due to sampling errors mayoccur. None of the limits for water are pass/fail limits. All limits are action limits. When action limits are exceeded the firm must investigate the cause of the problem, take action to correct the problem and assess the impact of the microbial contamination on products manufactured with the water and document the results of their investigation. With regard to sample size, 100 - 300 mL is preferred when sampling Water for Injection systems. Sample volumes less than 100 mL are unacceptable [73].

In its "**Microbiological Attributes of Pharmaceutical Ingredients and Excipients, Drug Substances and Non-Sterile Dosage Forms**" the USP in its Open Conference on Microbiological Compendial Issues (1996) proposed microbial limits for non-sterile dosage forms based upon the route of administration. For inhalants, the organisms of concern are *E. coli, P. fluorescens*, and *Salmonella species*; for vaginal applications, *E. coli, Staphylococcus aureus, P. Aeruginosa*, and *Candida albicans*; for nasal/otic/rectal/topical, *E. coli, Staphylococcus aureus, P. Aeruginosa*, and only for the nasal form, *Salmonella species*; for oral-liquid and oral-solid with synthetic ingredients or excipients, oral-solids with natural ingredient or excipients, *E. coli* and *Salmonella species* [8]. The corresponding aerobic total microbial counts permitted cfu/g or ml for each administrative route are <10, <100, <100, <100, <1000, <3000; the respective permitted yeast and mold counts are <2, <2, <10, <10, <100, <300 [8].

1.3 Chemical Quality

For US pharmaceutical applications the current standard is USP (United States Pharmacopoeia), which eliminated individual ion and metals levels in favour of conductivity and total organic limits (tables 1.3 and 1.5). Instead water systems are monitored to confirm they operate within their design specifications and produce water of acceptable quality. Action levels should represent quality concerns and the ability to effectively manage the treatment process (table 1.6). Conductivity and total organic carbon (TOC) are commonly measured online, and endotoxins and bacteria are measured offline. Methods for offline and online measurement are documented by USP [73].

Effective November 15, 1996, the former inorganic chemistry tests (for calcium, sulfate, chloride, ammonia, and carbon dioxide) were replaced with a three-stage conductivity test. The conductivity limit is pH dependent, but for example, at pH 7.0, conductivity should be less than 5.8 micro Siemen/cm. The former test for oxidizable substances was replaced with a TOC limit of 0.05 mg/L. TOC is an indirect measure of organic molecules present in water measured as carbon. The new tests allow continuous in-line monitoring of water using instrumentation rather than lab work [37].

Regardless of the system used to generate high purity water, under Federal regulations 21CFR 210 and 211, it must be validated. One of the primary references used in the validation of high purity water systems is the Parenteral Drug Association's Tech- nical Report No. 4, "Design Concepts for the Validation of a Water for Injection System. Validation often involves the use of an appropriate challenge. In this situation, it would be undesirable to introduce micro-organisms into an on-line system; therefore, reliance is placed on periodic testing for microbiological quality and on the installation of monitoring equipment at specific checkpoints to ensure that the total system is operating properly and continuously fulfilling its intended function [73].

Parameter	Upper control Limit
Resistivity [MΩ cm]	>17.5
Bacterial [germs/ml]	
• Life Milipore	200
• Total EPI	1000
• Total SEM	1000
• Pyrogene [ng/l}	2.5
DOC [ppb]	≤10
Ionic contamination [µg/l]	
• Cations (ESA/ICP0	5 (each)
• Anions (IC)	1 (each)
• Na	1
• Si	1
Particles [count/l]	
• OLC (0.5 µm)	500
• SEM (0.5 µm)	2000
(0.2 µm)	10000

Table 1.6. Quality of high purity water

1.4 Pretreatment and Protection

To improve the performance and to protect the purification equipment, pre-treatment is of vital importance to every water system. Pre-treatment encompasses all measures taken to improve the quality of plant water through its deionisation. Water, the universal solvent, dissolves or carries impurities from nearly everything it touches. Source water may contain a variety of impurities including dissolved minerals, dissolved organics

(including pyrogenic substances), and suspended solids such as bacteria, spores, colloids, and other insolubles. These impurities can cause processing and product problems ranging from equipment scaling or fouling (with corresponding high maintenance and operating costs) to odours, unacceptable tastes, discoloration, and bacterial spoilage. Configurations of water pre-treatment systems are similarly diverse; varying according to the nature of the impurities they are designed to remove [22].
As an important consideration in the system design is the need to define the quality acceptance and performance criteria for each component. The main goal of any water purification system design should be to properly apply and operate each component to achieve a system, which produces the intended volume of water product, at the specific quality, and in a reliable and economic manner [27].

It should be recognized that a purification system is essentially a grouping of components arranged in such a fashion so as to process the quality of feedwater into a product of predetermined quality. Each component within the system has one of several specific functions:

- To protect other components of the system from damage or non-function.
- To provide economical water pre-treatment prior to processing to final quality.
- To provide processing to the final quality.
- To recycle water or restore quality to former levels of purity.

If equipment selection is approached with the above in mind. Specifications, operational procedures, monitoring and preventive maintenance procedures will become a natural progression and allow for present or future validation [27].

Deionoization is the most widely method for producing high-purity reagent grade water. This process removes the ionic contaminations from the water.

Distillation is the broadest contaminant-removal technology. This process removes the contaminants from water. A combination of water purification techniques must be employed to remove all the impurities from water. Filters made of synthetic fibres (like multimedia filters) or activated carbon (or a blend of the two) are the predominant methods of point-of-entry water purification [27].

Reverse osmosis is more commonly used for pre-treatment of high purity water systems. Ultraviolet oxidation controls organics and bacteria, and it is often used in concert with filtration. Filtration can be employed as pre-, post-, or ultra-filtration. All have merits for removing particles, bacteria, and pyrogens. Adsorption is typically used to remove organics, chlorine, and ozone. Before source water reaches the plant, it has usually been rendered potable to EPA drinking water standards by several primary treatment steps such as flocculation, setting, sand filtration, and/or chlorination. Of course, some of these steps may take place within the plant as part of the pre-treatment system. Ultimately, all forms of USP water must be made from potable water (drinking water) [22].

1.4.1 Filtration: Filtration is the most commonly used means of cleaning up water. Activated carbon filters and reveres osmosis filters are predominant. A varity of fibre filters are also used. Some filters employing carbon are also made, but these should be avoided since there have been water absorption problems with the powder, complete with the contaminant it has filtered out, and passing into the water that is consumed [27].

Membrane filters are classified as surface or screen filters that are capable of retaining micro-organisms and particles on the surface of the filter or within a depth of 10-15 μm. Pre-treatment may consist of slow sand filtration preceded by Ozonation and biological activated carbon filtration [73].

1.4.1.1 ***Carbon filters/Turbidity filters*** are used to remove suspended particulates such as sand, silt and clay from the water. This will in turn enhance the performance of downstream equipment by removing the larger materials before the water reaches the fine stages of filtration. Using turbidity filters prevents downtime associated with fouling of the reverse osmosis membranes. Carbon Filters provide a high surface area and pore size distribution efficient for removal of a broad spectrum of organics. **Granular activated carbon** is made from select grades of bituminous coal. These filters also remove chlorine, which can damage types of reverse osmosis membranes [73].

Carbon filters often represent the most server source of microbial contamination. They are effective in removing chlorine and hydrocarbons and are somewhat less effective in removing heavy molecular weight organics common to surface water supplies, such as humic acid. These filters are often used in pre-treatment systems to minimize irreversible organic fouling of the deionising resins. When bacteria levels are measured at various points in a system, the highest levels are often found after the carbon filter because the residual chlorine is removed in the top portion of the bed, while the remainder of the bed provides a moist area with abundant carbonaceous material to support bacterial growth [21].

With an activated carbon filter; water passes either granules or a solid block of carbon. Although the carbon does physically filter out some of the large impurities suspended or dissolved in water, it is effective primarily in removing much smaller, and often more dangerous impurities. No other filtration method is as effective as activated carbon in removing many of the extremely toxic organic chemicals often found in water. Activated carbon removes more than the toxics than other filters, and does it better. Particles are removed as the water passes through the carbon by a process called *adsorption*. Activated carbon is extremely porous; approximately one of the materials has a surface area of one

m^2. This honeycomb of minute pores attacks and traps pollutants passing through it; the impurities are adsorbed [27].

1.4.1.2 Ultrafiltration (UF) Ultrafiltration is employed to minimize endotoxins in those drug substances that are administered parenterally. However, for most inhalation and ophthalmic products, purified water is used in their formation.
Membranes that do not have the capability selectively to removal salts, but act as filters for large organic molecules, can be considered ultrafilter membranes or molecular filters. While a microporous membrane filter removes particles according to pore size, an ultrafiltration (UF) membrane functions as molecular sieve that separates dissolved molecules on the basis of size by passing a solution through an infinitesimally fine filter (1000-100,000 molecular weight, MW. The UF is a selective permeable membrane that retains most macro-molecules above a certain size, including colloids, microorganisms, and pyrogens. Smaller molecules, such as solvent and ions pass into the filtrate [27].

1.4.2 Water Softeners are used to remove hardening minerals, such as calcium and magnesium. An over abundance of these minerals can cause scaling of reverse osmosis membranes. Through an ion exchange process, the resins remove hardness causing cations and replace them with sodium, which, not impart damage to membranes. Softening resins are high capacity synthetic cation exchange resins for maximum regeneration efficiency [73].
Softeners are needed when mineral content (especially Ca^{++} and Mg^{++}) of the source water is substantial. If the mineral level is moderate, softened water is sometimes needed only for the regenerates used in the cation/anion exchangers. In either case, of course, microbial contamination of the softener affects the rest of the system. Softeners are generally more vulnerable to microbial contamination than are cation/anion exchange systems, because the latter are periodically regenerated with bacteria killing

acid or alkali. Sodium chloride regenerating solutions for softeners (brine) will not provide the necessary periodic bacteria kill. Consequently, situations can develop in which microbial population steadily increases in the bed over the course of its operating life. Some bacteria will be removed or reduced during a backwash operation performed in the regeneration process, but many softeners are designed with minimal freeboard (headspace) and with insufficient backwash flow. This is particularly true with smaller units (that is, those with flows of less than 20 gpm). Thus, although the softener is usually deployed at an early stage in the system frequently even in a different building from the deionisers, it must be recognized as a probable contributing source to an observed microbial problem [21].

1.4.3 Chlorination

The addition of treatment chemicals to water systems for the control of biological fouling was first suggested in England around 1827. At that time relationship between micro-organisms and fouling – or disease – was unknown. In 1894, Traube determined the purifying properties of chlorine compounds in water treatment. Since then, such chemical compounds have remained the most widely used in the treatment of biological fouling, and chemical treatment is still the preferred method for the majority of biofouling problems in purified water systems and other industrial water circuits [23].

1.4.3.1 ***Chlorine*** as with most other treatment chemicals may be applied in different concentrations and forms to effect varying degrees of purification. Depending on the application and the degree of control desired, chemical treatments are known by a variety of terms, including biocides, disinfectants, sanitizers, germicides, antibiotics, antiseptics, sporicides, and bactericides. Although antibiotics and antiseptics are also used in the control of micro-organisms, they are typically applied to living tissues, not water systems. Similarly, sanitizers and sporicides are normally applied to static surfaces.

Confusion over the definitions associated with chemical treatments can result in mis-application [23].

When preparing water for use in pharmaceutical manufacturing, the antimicrobial agent chlorine, if present, is usually removed by a water pre-treatment system such as a carbon filter. In the absence of chlorine, subsequent water streams become vulnerable to microbial invasion, and microbial growth can occur under certain circumstances [21].

Differences between various treatment classes often depend on dosage and application rather than specific chemical types. Regardless of whether the application is for medical or micro-electronic processing operations, the complete removal of all organisms from purified water is the desired result. Therefore, biocides are usually the preferred chemicals for the treatment of biofouling [23].

1.4.3.2 Biocides agents that prevents the growth of bacteria and their spores. The use of Biocides is the most common approach to biofouling problems in industrial water systems is the use of biocides, or, more correctly, microbiocides. Sometimes they are referred to as disinfectants (Noss CI, Isaak RA, 1990), but this might be misleading, because an industrial system will not be disinfected, i.e. cleared from pathogenic microorganisms (Wallhauber KH, 1988); in the best case, the total number of cells in the water will be drastically reduced [31].

1.4.3.3 Hydrogen Peroxide A commonly employed purified water system biocide can also be readily degraded by ultraviolet light. However, it has relatively little activity against attached bacteria in biofilms. There is also evidence, which suggests that hydrogen peroxide requires catalytic concentrations of Fe^{++}, Ni^{++}, or Cu^{++} for optimal biocide (Block, 1983).

Surfactants such as the quaternary ammonium compounds have excellent biocide activity in addition to their intrinsic detergency. Synergistic combinations of chlorinated or brominated compounds with surfactants may provide additional activity against bacterial biofilm populations. Their ability to interact with surfaces, however, creates problems related to removal of these compounds following application. Larger volumes of rinse water are required for the surfactant compounds, than, for example, hydrogen peroxide usage [25].

1.4.4 Ion-exchange

Demineralization, deionisation (DI), or ion exchange is based on the removal of impurities from the water by means of synthetic resins that have an affinity for dissolved ionised salts. Most synthetic resins are made of styrenes cross-lined with divinylbenzen before being activated with either sulphuric acid to make them cation acceptors or amines to make them anion acceptors. A DI system might be a tow bed, multiple beds, or mixed bed type depending on raw water conditions and water quality required. Demineralised water systems are tailored to fit the end use, e.g. feed water to a still, USP (United States Pharmacopoeia) purified water, point of use polishing, de-acidification, de-colorising silica removal, high pressure boiler feed, carbon dioxide removal, and oxygen removal. RODI is a system using RO followed by DI to produce high purity water [27].

1.4.5 Method of water pre-treatment system

Supply water from wells or a municipal water supply is passed through a carbon filter to remove chlorine and the lighter weight hydrocarbons. Next, if the supply water is sufficiently hard, it may be passed through a softener or demineraliser to remove hard cations like Ca^{++} and Mg^{++}, replacing them with sodium (Na^{+}) to form more soluble

salts. Finally, the softened water is passed through a deioniser, which replaces cations with hydrogen ions (H^+) and anions with hydroxyls (OH^-). The deioniser might consist of a mixed-bed and/or a two cation-anion exchangers resin system. Frequently, some of a filter or strainer is deployed at the deionser's outlet point to remove large particulate, such as the resin fragments that might originate with ion-exchange system. The pre-treated water is then collected in a holding/distribution (HD) system, where it may be stored until needed. As is the nature of such things, innumerable variations of this water pre-treatment system exit [22].

Pre-treatment systems may involve several varieties of two-bed and mixed-bed cation-anion exchange units. The two-bed system is generally simpler and less expensive to regenerate, but mixed-bed units provide more efficient deionisation by offering many minute stages of cation-anion exchange. One frequent practice in the industry combines the features of both by using mixed-bed units as a polishing system after passage through a two-bed system, see figure1.4 [22].

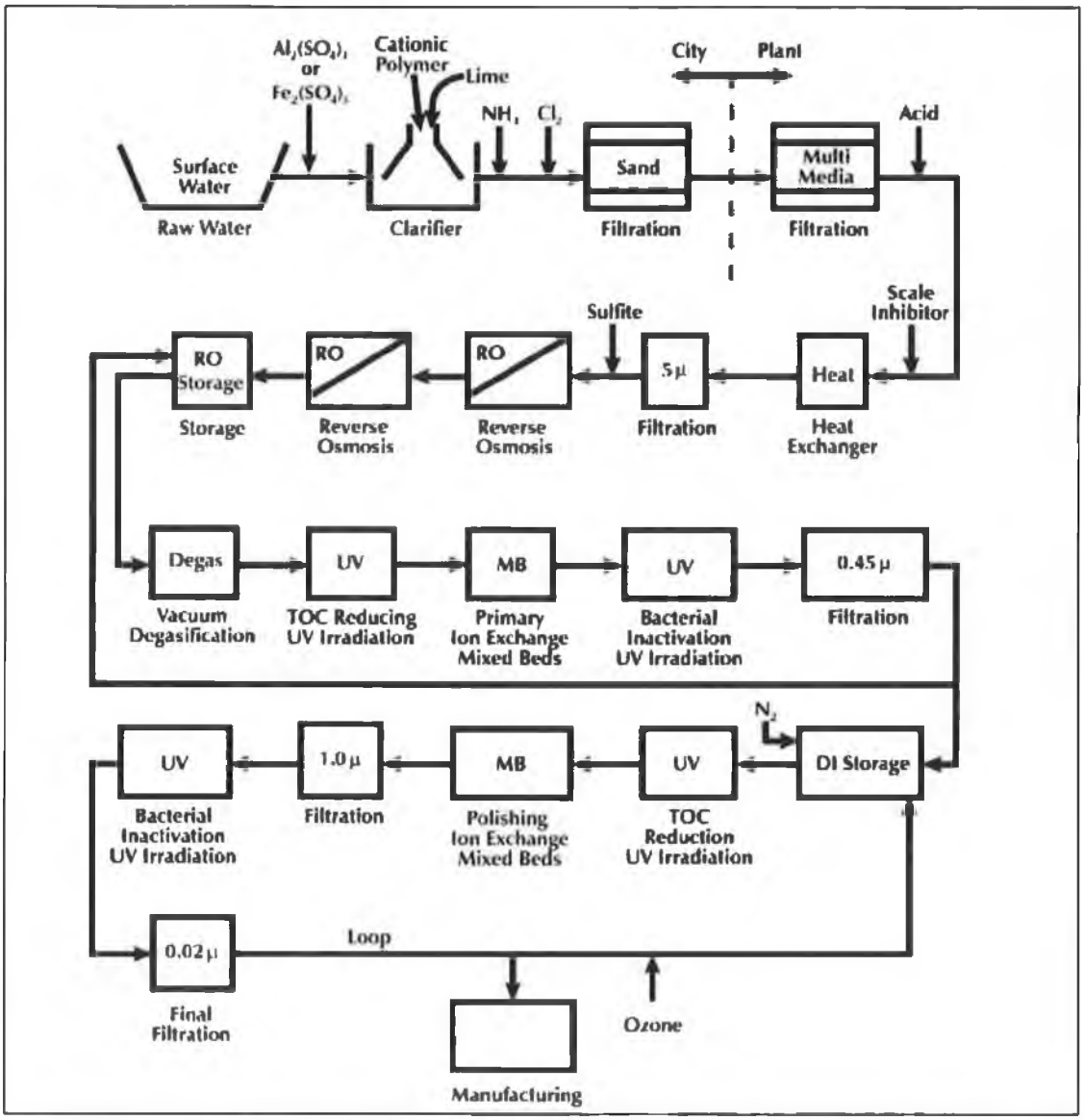


Figure 1.3. High purity pre-treatment system

For large pre-treatment systems, it is common to deploy **twin** two-bed systems, following each with a mixed-bed polishing step. Similar redundancy is occasionally practiced in almost any step of a pre-treatment system when warranted by special capacity or unique quality concerns. It is helpful to note, that compressed air can be a

source of microbial invasion. Because it is used in the regeneration of mixed-bed deionisers and may be used in degasification (for removal of H_2S, CO_2, NH_3, and so on) and in pressurization systems, both the source and quality of compressed air merit careful review [22].

1.4.6 Final treatment and polishing

Supplementary design considerations should provide purity water at 10 to 18 MΩ.cm qualities; however, 0.5 to 10 MΩ.cm qualities are probably acceptable for most non-WFI services. Of prime importance are the special provisions required to ensure and maintain low 'bioburdens' and the high degree of reliability needed, i.e. 7 days per week, 24 hours per day continuous operation without shutdown. With proper attention to design, four shifts of operation required less than one shift of operator coverage in one particular plant. Some high purity water systems operate in the lower ranges of purity, i.e. 0.1 to 5 MΩ.cm [27].

1.4.7 Reverse Osmosis

Reveres osmosis (RO), ultrafiltration (UF), and electrodialysis, are characterized as material separation processes, which play an ever increasing role in water high purity treatment, through separates dissolved substances or/and finely dispersed particles from liquids [1]

RO is an advanced unit operation in water purification, capable of removing at least 90% of dissolved solids, organics, bacteria, and other impurities in water. RO has used as a pre-treatment for ion exchange demineralisation by industry, and has application in waste treatment, chemical separation, food and drug processes. Reverse osmosis is used to, remove a broad range of contaminants from surface water (River water) such as

DBP-precursors, synthetic organic chemicals such as pesticides, hardness constituents and salts [5].

Desalination plays an important role in producing pure water from brackish water (sea water), reverse osmosis is by far the most efficient way to remove colloidal and dissolved silica, which can be found in high concentrations in brackish water [4].The aim of RO is to realize a stable operation. A stable operation can be characterized as one with a very low decrease of the mass transfer coefficient and a low increase in differential pressure, resulting in a low cleaning frequency of the membrane units [6].

Reverse osmosis is an effective technology to remove organic compounds from water bodies, especially for those that contain low concentration and low molecular weight organic compounds. A traditional RO membrane is limited due to high operational cost and maintenance as RO involves requirement of high pressure to the system and the need for extensive pre-treatment. Recently, new generation ultra-low pressure reverse osmosis (ULPRO) membranes were introduced. This kind of membrane can be operated at very low pressure. Most ULPRO membranes are multi-layer thin film composites of polymers. The active membrane surface layer usually consists of negative charged sulphone or carboxyl group. Due to the active surface layers, ULPRO membranes have improved fouling resistance against hydrophobic colloids, oils, proteins and other organics. In order to increase water flux, a charged hydrophilic layer is attached to a hydrophobic (UF) support membrane. This makes the membrane favourable for the orientation of water dipoles. Generally, the flux is, inversely proportional to the membrane thickness. ULPRO membranes normally have a corrugated skin surface that can improve flux significantly. In low operating pressure, ULPRO produces specific flux of more than 60l/m^2h MPa. This flux rate is about double the flux of previous generations of composite RO membrane. ULPRO membrane has been shown to be energy saving, and is efficient in rejecting organic and inorganic species as compared to the convential RO membrane. Many studies have been devoted to rejection of organic

compounds by RO membrane. It was under stood that the removal of organic compounds by RO is governed by mutual interactions between membrane material and solute molecules. Hofman et al. reported that the rejection strongly depends on the membrane material and solute structure. The increasing size of the side chain results in a higher rejection by the low-pressure reverse osmosis membrane. Kiso et al. developed the relationship between the rejection efficiency and molecular width by using cellulose acetate RO membranes. Many researchers found that the solute charge affects the removal efficiency in uncharged RO membrane [7].

1.4.7.1 Osmosis Is defined as the spontaneous passage of solvent from a dilute solution to a more concentrated solution across an ideal semipermeable membrane, which allows solvent (water) flow but impedes transport-dissolved solids (solutes). The solvent continues passes from one side of the membrane to the other of more concentrated solution by **Diffusion Mechanism,** see figure 1.6, until the pressure is large enough to prevent any net transfer. At a certain pressure, the amount of solvent which passes in each direction, is equilibrium; This pressure is defined as the osmotic pressure of solution. It is considered as the pressure between the nature of the solution and the pure solvent. If the pressure is greater than the osmotic pressure of the solution the flow reverse. Pure solvent flows from the more concentrated solution to the solvent, this phenomenon defined as reverse osmosis [2].

Several osmotic pressure measurement methods are available. It can be calculated from vapour pressure lowering measurements, from freezing point depression data, from direct measurement, and by the equivalent of the ideal gas law equation.

The difference between the operating pressure and the osmotic pressure is a driving force for operation of a reverse osmosis unit. Recovery must be constant and coincide with a variation in concentration.

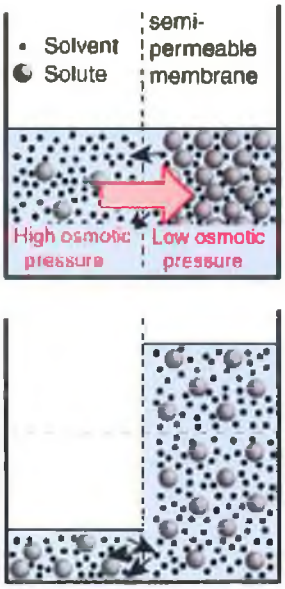


Figure1.4.The osmotic pressure

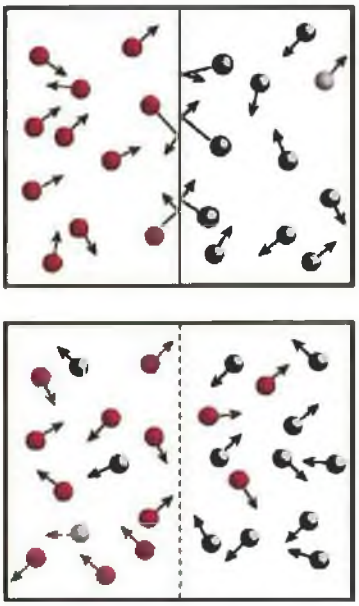


Figure1.5. Diffusion mechanism

1.4.7.2 Membrane problems to prevent high-pressure feed or concentration into the low-pressure product, a reliable seal is employed. To minimise concentration polarisation and fouling, a controlled feed and concentration flow path is required. Product flow through a membrane is directly proportional to the surface of the membrane and inversely proportional to the thickness. A module design containing the greatest possible area of the thinnest possible membrane offers the advantages of low-pressure vessel cost and smallest required RO plant size.

1.4.8 Distillation

There are some proponents of the production of high purity water who include distillation, and some proponents for whom distillation is the only purification step for high purity water. Distillation is a process that removes water from its impurities by evaporation. In this phase change process particulate matter, dissolved organics, bacteria, viruses, pyrogens as well as dissolved gases including carbon dioxide and oxygen are removed [27].

This is proven and acceptable for producing water that is suitable for injection meeting the requirements of USP23. It is the only single process capable of producing water free of organics, ionisable, bacteria, particles matter, and dissolved gases. These are the major disadvantages of producing high purity water by distillation:

- The cost per-liter of water produced can in many instances be substantial, because of the latent heat of evaporating.
- Multiple effect destination can be expensive but operating costs must be balanced with running cost.
- Maintenance can be severe if proper pre-treatment equipment is not utilized to protect the heat exchanger system from scaling.

All stills must be designed so that the water vapour phase separates from the liquid phase without carrying over particulate contamination as entrainment [27].

Failure of a still to prevent entrainment can mean contamination of its distillate with pyrogenic endotoxins derived from the cell walls of gram-negative bacteria in the still's feedwater. This, of course, would be unacceptable to produce of parental products, which rely chiefly on distillation for purification of water for manufacture. Most modern stills will thermally inactivate the micro-organisms and will depyrogenate water by separation, but some will not. Certain thermal compression

stills, for instance, operate at too low temperature to provide the lethality required to kill thermal stable micro-organisms. Furthermore, some single-effect stills, particularly those without continuous blow-down, have been shown to entrain endotoxins, Finally, any still can be flooded (that is, boiling liquid can flood into the vapour system) if it is improperly operated [21].

1.5 Deionized Water (DI)

1.5.1 Ultra pure water Used in semiconductor manufacturing; produced by removing all ions of dissolved minerals using reverse osmosis and ion-exchange systems; DI water should also be free particles, bacteria, organics, and dissolved oxygen; purity of deionised water determined based on its resistivity; target resistivity 18 magaohm-cm. DI water (figure 1.6) is the most prevalently used material in micro fabrication process, and is used mainly for rinsing and cleaning of wafers. In order to achieve the quality and purity levels required by modern micro-fabrication processes deionised must be manufactured on site. It usually takes 4-6 gallons of raw tap water to produce one gallon of deionised water acceptable for use in micro-fabrication. It is import that deionized water is continuously re-circulated in order to achieve the quality and purity levels. It is also important that purity is continually monitored.

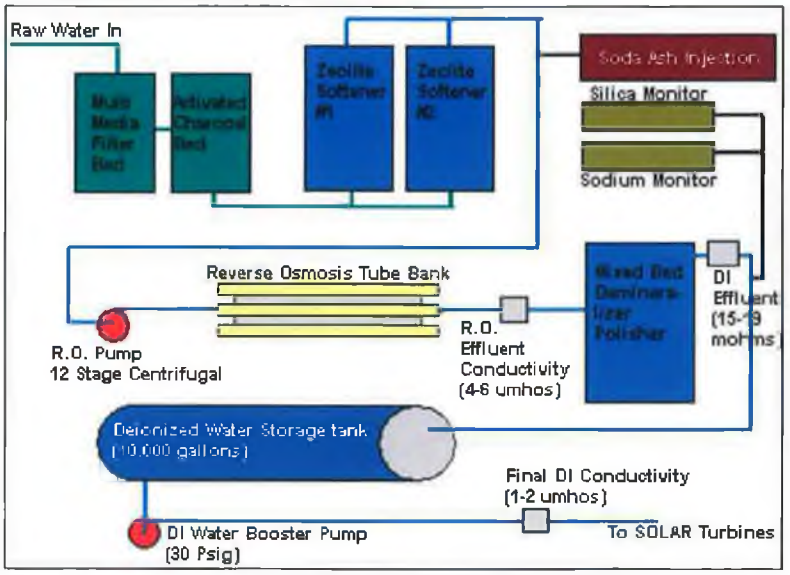


Figure 1.6. Deionised water system

Ultra pure water is produced in the utility area and distributed to clean rooms through piping of several hundred meters. In clean rooms, the main stream is divided into branches and finally supplied to many points of use. Because the piping is the final material that the purified water contacts, secure maintenance of water purity is required in the piping system between the ultrapure water production systems and the point of use.

The quality of ultrapure water decreases due to various causes while passing through long and complicated piping. Possible causes are dissolution and/or desorption of impurities from the piping wall, propagation of bacteria, and penetration of air and gases into the inside of the piping.

The use of purified water in various industrial and medical applications has increased dramatically over the past twenty years. These different applications often require varying levels of water quality. Each industry sets specifications for the acceptance of purified water quality based upon their product or process demands. The aggressive nature of ionically ultrapure water (18.2 MΩ. cm) can complicate its use in, for

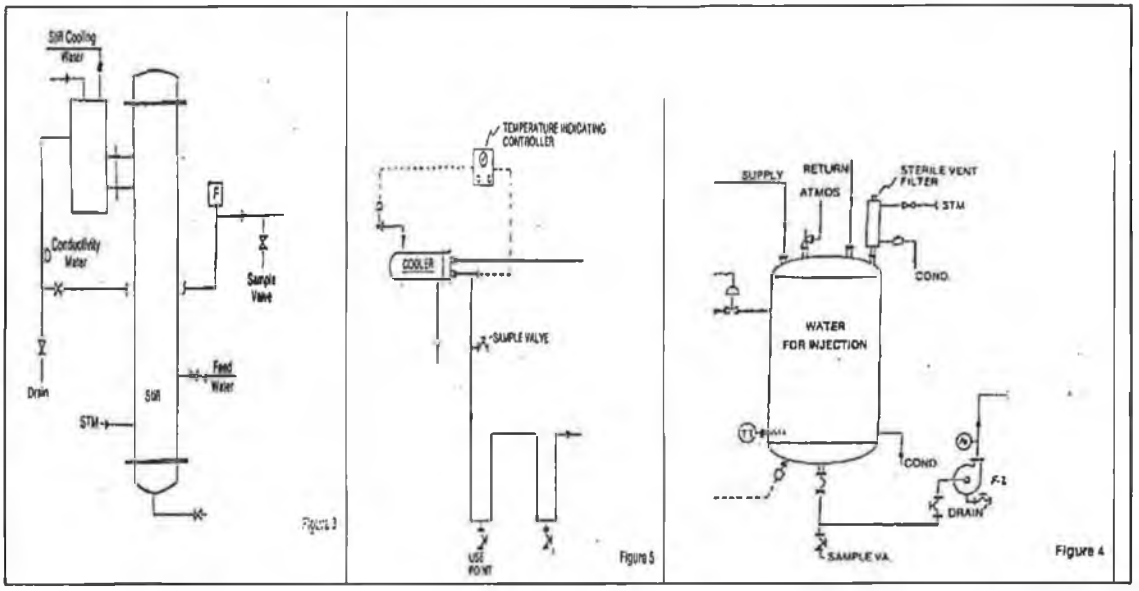


Figure1.8. Water For Injection System (WFI)

USP Purified Water may also be purified by "ion-exchange or other" mechanisms. The only difference in compendial quality between the two grades is that WFI (figure 1.8) must meet a pyrogen test; it need not be sterile, but it must be produced, stored, and distributed in such a way that micro-organisms, if present, do not produce pyrogenic substances. [21].

Although USP23specifies no microbial limits for either Purified or WFI grades of USP water, the Pharmaceutical Forum recently suggested possible total microbial (aerobic) limits as presented in table 1.7 (no reference is made to the type of contaminants involved other than the coliforms):

Water type	Microbial limit
Drinking Water	500 cfu/ml
Purified Water	100 cfu/ml
Water for Injection	50 cfu/ml

Table 1.7. USP microbial limit of water

CHAPTER 2

THE DISTRIBUTION SYSTEM

2.1 Distribution

The high-purity water, after production, must be piped and distributed to its areas of use, often several hundreds of meters away. There may also be numerous points of use. What is desired of the piping is that it be inert to the water it conveys at each and every stage of the system, that it does not leach any of its components into the water, and that it withstands the sanitizing reagents periodically used to combat the biofilm inevitably formed upon inner surfaces. The piping must be of a composition that will permit its erection in sag-proof dispositions, so as to prevent the formation of stagnant pools. The piping must also be impervious to its outside environment, so as to have a long service life. It should not permit the permeation of air or other gases, nor of ultraviolet or other radiation. All of the above requirements should be met, with due regard to economic considerations including costs of installation and maintenance. Above all, the piping must preserve the purity of produced water being conveyed by it [8].

One of the basic considerations in the design of a system is the type of product that is to be manufactured. For parenteral products where there is a concern for pathogens, it is expected that Water for Injection will be used. This applies to the formation of products, as well as to the final washing of components and equipment used in their manufacture. Distillation and RO filtration are the only acceptable methods listed in the USP for producing Water for Injection. However, in the bulk Pharmaceutical and Biotechnology industries and some forgein companies, Ultra Filtration (UF) is employed to minimize endotoxins in those drug substances that are administered parenterally [17].

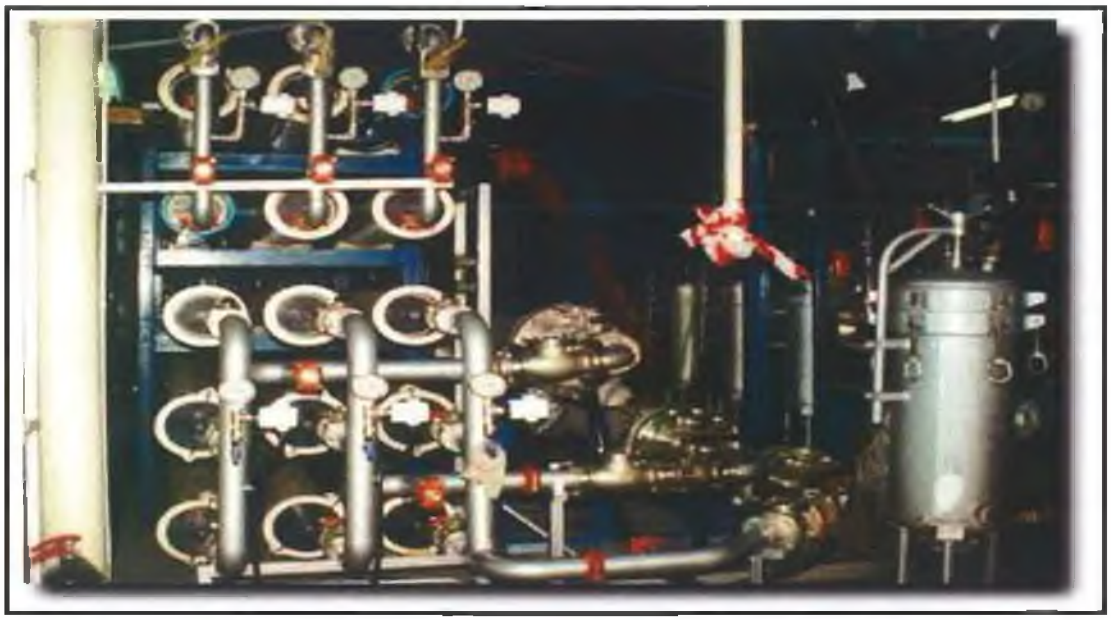


Figure 2.1.High Purity Water Distribution System

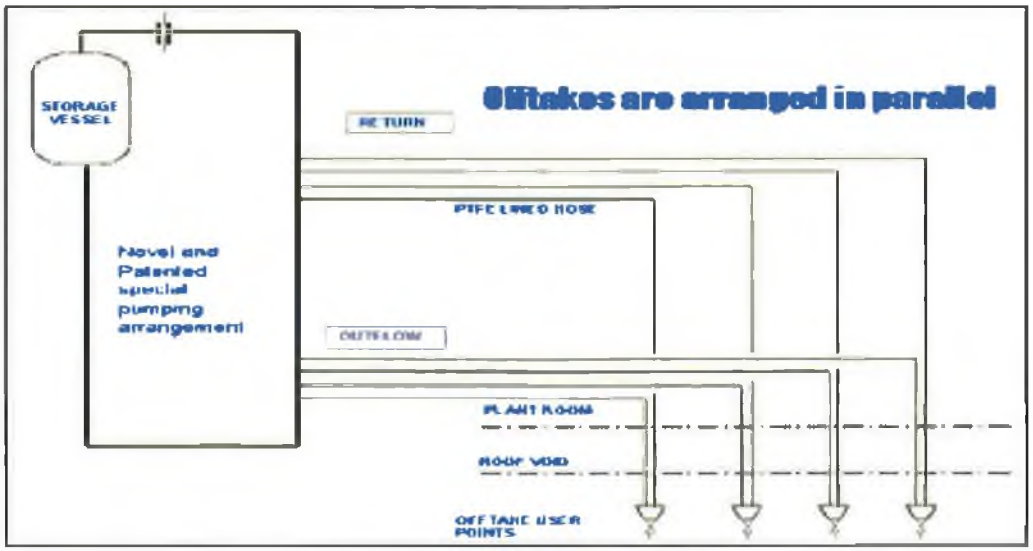


Figure 2.2. Distribution Systems and points of use

Another design consideration is the temperature of the system. It is recognized that hot (65-80°C) systems are self-sanitizing. While the cost of other systems may be less expensive for a company, the cost of maintenance, testing and potential problems may be greater than the cost of energy saved. Whether a system is circulating or one-way is also an important design consideration. Obviously, water in constant motion is less liable to have high levels of contamination. A one-way water system is basically a "dead-leg". Finally, and possibly the most important consideration, is the risk assessment or level of quality that is desired. It should be recognized that different products require different quality waters. Parenterals require very pure water with no endotoxins. Topical and oral products require less pure water and do not have a requirement for endotoxins. The quality control department should assess each product manufactured with the water from their system and determine the microbial action limits based on the microbial sensitive product. In Lieu of **stringent** water action limits in the system the manufacturer can add a microbial reduction step in the manufacturing process for the sensitive drug products [17].

The quality of ultrapure water decreases due to various causes while passing through long and complicated piping. Possible causes are dissolution and/or desorption of impurities from the piping wall, propagation of bacteria, and penetration of air and gases into the inside of the piping. In order to suppress the degradation of ultrapure water, it is important to select piping material of low leachables, and to prevent the propagation of bacteria by suitable disinfection. Manufacturers of piping, manufacturers of ultrapure water production systems and users of ultrapure water have done their own method of selecting piping materials. Because standardizing the test procedure for evaluating piping materials is very important for ultrapure water technology, test procedures were established with the cooperation of ultrapure water production system manufacturers in Japan. Yoshito M. and Koichi Y. (1991) introduced standardized test procedures and examples of test result. The established test procedure basically consists of sealing the ultrapure water in short pipe length for a certain period; this is followed by an analysis of this water. Historically, various materials have been put into use for

ultrapure water distribution piping as follows: poly-vinyl-chloride (PVC), poly-propylene (PP), clean-PVC, poly-vinylidene-fluoride (PVDF) and poly-ether-ether-ketone (PEEK), were used as heat resistant piping materials. PVDF and PEEK piping are used for the purpose of supplying hot ultrapure water or sterilizing the distribution systems with hot water. PVC and PP contain additives such as stabilizers and pigment, while PVDF, PFA and PEEK have no additives. In these materials, PEEK is the best engineering plastic material and contains no specific elements such as chloride ions; also, PEEK has the mechanical strength closer to that of metals. Clean-PVC release chloride (CL) and calcium (Ca) ions into water. Because the chloride ion is a main constituent of PVC, it will be difficult to suppress the amount of chloride ion release. In the case of conventional grade PVC, it is known that a large amount of lead (Pb) is leached out into water. Sodium (Na), calcium (Ca) and sulfate (SO_4) ions were detected. Amounts of the ions remained low at 25°C, although, at elevated temperature dissolution of sodium and sulfate ions increased several times. These ions are considered to be components of additives. As the amount of dissolved impurities did not increase when immersed for longer periods, dissolution is believed to be completed at the early stage immersion [36].

Piping in WFI systems usually consists of a high polished stainless steel. In a few cases, manufacturers have begun to utilize PVDF (polyvinylidene fluoride) piping. It is purported that this piping can tolerate heat with no extractables being leached. A major problem with PVDF tubing is that it requires considerable support. When this tubing is heated, it tends to sag and may stress the weld (fusion) connection and result in leakage. Additionally, initially at least, fluoride levels are high. This piping is of benefit in product delivery systems where low-level metal contamination may accelerate the degradation of drug product, such as in the Biotech industry [17].

The FDA permits plastic piping, despite its having been proscribed in GMP/LVP 212.49. However, it must be validated with respect to extractable, and its method of

being joined must not yield crevices and must withstand the necessary elevated temperatures. [8].

2.1.1 Stainless steel the overwhelming choice as the material of construction for high purity water delivery system is 316L stainless steel. However, careful attention needs to be directed to welding procedures, polish, cleaning, passivation, inspection, and documentation. The use of sanitary fittings is the preferred approach to joining components where access is required. The use of compression fittings should be avoided b ecause t hey c ontain l arge c revice a reas t hat m ake c leaning a p roblem a nd could advance the growth of bacteria. Flanges are not generally recommended because of alignment difficulties leading to an imperfect joint. Valves should be of the sanitary diaphragm design mechanically polished to at least 180 grit followed by electropolishing. E lectropolish p rovides s everal benefits; it r emove p eaks, r ound o ut regularities, improves cleaning by creating a smooth finish, passivates the weld surface, and improves the inspection process by highlighting imperfections in welds. The use sanitary fittings are strongly recommended throughout [27].

Composition of the steel is significant in at least two respects. First, it must be capable of withstanding corrosion; and second, it must not cause changes in resistivity properties consequent to being welded. For high-purity water and pharmaceutical-product installations, concerns with materials of construction centre largely on corrosivity. Because of their relatively high resistance to corrosion, the stainless steels are used in the fabrication of stills, tanks, and their interconnecting tubing. For a stainless steel grad, the designation L signifies a low carbon content, particularly desired where welding operations are to be employed. To minimize the opportunities for carbide precipitation, the amount of carbon is restricted in the L-grade stainless steel formulas. Both 304 and 316 can contain as much as 0.08% carbons. Their L grades can be composed of less than 0.03%. Other properties of the stainless steel are also affected, some adversely so, by the formula changes involved in the lowering of the carbon content. The chief difference between 316 and 304 is the molybdenum content (about

2.5% in the 316 grade). Molybdenum is regarded as conferring greater corrosion resistance, as it minimizes chromium carbide formation. This facilitates the formation of chromium oxide, whose inertness is the object of using stainless steel. It may well be, whoever, that in particular contexts the resistivity of 304 stainless. In recent major pharmaceutical installation, 316 tubing was chosen for contact with deionised water, while 304 tubing was selected for the conveyance of distilled water. The reasoning was that 304 stainless steel considered more corrosion-resistance at low temperatures, while 316 stainless steel is judged to be better under hot temperatures. Tanks and tubing may be exposed to different temperatures. Usually, the tanks face a more demanding environment and are often fabricated from a higher, more resistant alloy. Vally and Rathbun (1977) recommended that 316L be used for all equipment surfaces that come in contact with deionised or distilled water. However, neither opinion nor practice is unanimous in the industry. It is said that 316L stainless steel has better resistance to pit corrosion than dose 304L, and that the latter is more difficult to weld. More importantly 304Lis not slandered in all sections. The use of two differing metals can more likely be avoided therefore if 316L is used because valves and other parts are more readily available in this composition. The mixing of metals is believed to cause corrosion. In short summary, 304 stainless steel is corrosion resistance enough at low temperatures. Higher temperature levels require the 316 grades. The advantages and disadvantages of the polymeric and stainless steel piping is shown in Table 2.1.below [8].

The Material	Advantage	Disadvantage
(1) Polymeric piping	▪ Minimizes opportunities for heavy-metal pickup by the water. ▪ May offer some advantages for piping and tank constructions.	▪ Is not strong. ▪ Requires the installation of much more support to prevent the pipe sagging that would lead to poor drainage and difficulty in cleaning. ▪ Plastic piping may require being supported every 4 or 5 feet, this can double the installation costs.
(2) Stainless steel	▪ Stainless steel piping is supported every 8 to 10 feet. ▪ Has the advantage of already being in place in a conservative industry prudently cautious to change.	

Table 2.1.The advantages and the disadvantages of the polymer and stainless steel piping.

2.1.2 Polyvinyl chloride the term "polyvinyl chloride" refers to a polymer; essentially it is a polyethylene, every alternating carbon atom of whose chain bears a single chlorine atom substituent. The chlorine groups confer some oxidative stability over the corresponding hydrogen substituents. However, the chlorine atoms, hydrogen atoms, to form hydrogen chloride. The dehydrochlorination reaction introduces color-producing unsaturating in the remaining polymer. The polymer is compounded with stabilizers, often organometallic, in nature, to protect these molecules against dehydrochlorination: with activated carbon, to confer better mechanical properties of rigidity and impact

strength and to protect against UV light; with fillers to add opacity and color; and with plasticisers for formability. Indeed, PVC cannot survive most modling, extruding, or other forming processes without the addition of stabilisers against dehydrochlorinations. What actually used in piping fabrication is not PVC polymer but PVC compound. Because PVC piping may contain, and hence leach, the added compounding materials, other piping materials are progressively replacing it. The chief concern is the metallic (usually heavy metal) stabilizers and their leaching into the water. Stainless steel has largely replaced PVC in pharmaceuticals; PVDF has been and is replacing it in the semiconductor industry. Where PVC is still in used in the electronics industry, it is largely in the makeup portion of the water system. Once the water achieves high purity, it is usually conveyed in PVDF piping. When PVC piping is used, it should be constructed of high-grade polyvinyl chloride compound intended for quality pipe. It should contain no metal stabilizers and as little plasticiser possible. The pipe should be precleaned prior to its installation. It is only fair to state that some PVC pipe manufactures do believe they have demonstrated their product to be suitable for the conveyance of high-purity water. Sinha and Van winkle (1993) found extractable from such PVC pipe rinse out to equilibrium impurity levels in less than 30 days, even in the presence of additives and solvent from the pipe-joining process. The FDA in its "Guide to Inspections of High Purity Water Systems" (1993) emphasizes the concern about extractables: "Most of these systems employ PVC or some type of plastic tubing. Because the systems are typically cold, the many joints in the system are subject to contamination. Another potential problem with PVC tubing is extractable [8].

2.2 Piping system Dead-legs

One common problem with piping is that of **"dead-legs".** The proposed LVP Regulations defined dead-legs as not having an unused portion greater in length than six diameters of the unused pipe measured from the axis of the pipe in use. It should be pointed out that this was developed for hot 75-80°C circulating systems. Any unused

section of pipe connected to another pipe or conduit through which water is flowing may contain, depending upon the venturi effect caused by the flowing water, relatively quiescent or stagnant quantities of water. This nonflowing water is of concern because of the higher planktonic, organism counts found for waters. Such unused sections of pipe are called dead legs. The term has been extended to any section of non-flowing water during its period of stagnation, even if the stagnation is not continuous. Thus filter holders are sometimes characterised as dead-legs when containing water not in active flow. Transport into a dead-leg is not directly a function of recirculation velocity. With colder systems, any drops or unused portion of any length of piping has the potential for the formation of a biofilm and should be eliminated if possible or have special **sanitising** procedures. There should be no threaded fittings in a pharmaceutical water system. All pipe joints must utilize sanitary fittings or be butt-welded. Sanitary fittings will usually be used where the piping meets valves, tanks and other equipment that must be removed for maintenance or replacement. Therefore, the firm's procedures for sanitization, as well as the actual piping, should be reviewed and evaluated during the inspection [17].

The occurrence of dead-legs is perhaps the most common cause of bacterial contamination in water treatment systems. Michael Bukay write a few actual case histories of dead-legs he had found in water systems that he examined. He concluded that the dead-legs could be as subtle as a sample port or as blatant as a piece of equipment left to sit idle for months in the standby condition. There are many ranges of severity in between. Many times, especially in older buildings, dead-legs are located in entangled masses of piping somewhere in the ceiling or beneath the floorboards, and difficult to identify [9].

All dead-legs tend to increase the potential for stagnation pockets, which, over a period of t ime t end t o b ecome b ioburden b reeding grounds. D ead l egs s hould t herefore b e removed, or their effect minimized. Some acceptable rules have been developed for this purpose and some vendors have developed special fitting and/or valves to accomplish

this. One acceptable rule is the so-called '6D' rule where the straight run of the dead-leg from the centreline of the pipe, fitting or valve shall not exceed six times the smaller fitting diameter. On occasion, designers have used 5D, 4D and 3D rules for some applications [27].

To prevent the growth of bacteria in ultrapure water, it is said that it is very important to decrease "dead-legs" in p iping systems. However, the cause of bacteria de tection in ultrapure water not only increases in dead-legs, but also increases on the surface of piping. Recently, it was found that bacteria take nutrients from ultrapure water effectively by adhering to the wall with a sticky secretion [36].

The use of flush-diaphragm or sanitary valves is also important in eliminating the stagnant-water areas of dead-legs. It should be as vertical as possible to ensure the rapid drying of its inner surfaces. Prior to water being drawn at such a point of use, an adequate flushing of the dry pipe should be made. The potential for dead-legs usually occurs at occurs at valved branches. Special close welded branch valves have been devised that serve to eliminate or minimize such occurrences. Periodic dr aining and flushing can be effective practices in maintaining microbial control of the system. In this connection, the pipes conveying the water ought to be inclined about 1/16 to 1/8 inch per running foot (0.5 to 0.31cm per tuning 30 cm) to ensure through drainability .The FDA GMPs (1976) do not specific the pitch of the pipes, only that they should drain completely [8].

2.3 Storage and Reservoir

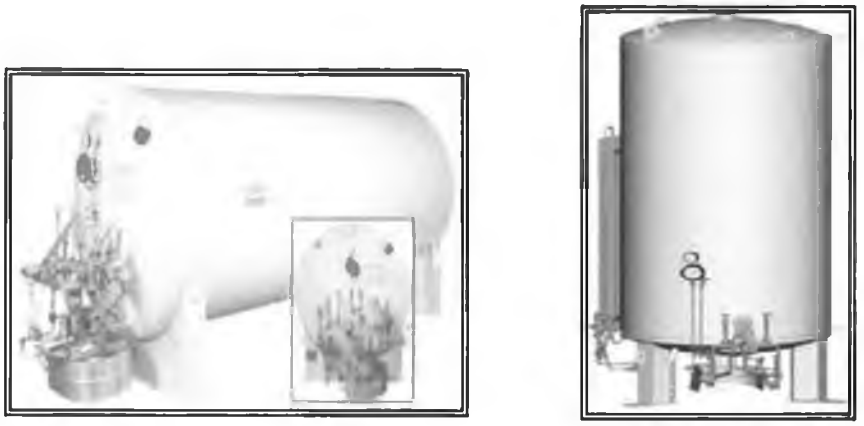


Figure 2.3. Storage tanks for high purity water

The HPW storage and distribution system, supplies the high purity water used throughout the plant to both batch and integrated rinsing equipment used in the preparation of packaging components and processing equipment that contact product formulations. Although derived from the same distillation source and held under the same storage conditions to maintain water quality, HPW is not batch and discretely tested, as is the batched WFI and is therefore not used in product formulating. However, this continuously replenished HDW system is routinely tested to as sure that the HPW water meets WFI USP specifications [30].

Water storage tanks (Fig. 2.3) are seen as contributing to the problem of organism proliferation. When they are not completely full, their walls are particularly susceptible to such growth. At least one designer of water storage tanks incorporates UV into the ceilings of the tanks. To minimize organism growth, the contents of water storage tank are circulated at rates calculated to approach two turnovers per hour. Large tanks may

be limited practically to less frequent turnovers. In one operation, a 10,000-gallon WFI tank turned over at the rate of 50 gpm, equivalent to 3,000 gph. Recalculation rates are normally determined by the piping system, with the actual tank recalculation being a resultant. The rate of liquid movement in piping is generally recommended as not less than 5 lineal feet per second. Tanks in a recirculatory mode often have the returning liquid sheeted down the tank sides by way of spray balls. Spray balls are also useful for dispensing cleaning and sanitising agents down the walls of the storage tank headspaces where microbial problems are inclined to exist. Unless the tank is rated to withstand a full vacuum, its vents must be sized to prevent the collapse of the vessel as its liquid contents are withdrawn. Rupture disks, as commonly used to prevent tank collapse, are relatively easy to design for tanks that can withstand some pressure or vacuum differentials. When, however, they are required for tanks built for use at ambient pressure, they must be calibrated with some precision, and that render them costly. The rupture of a rupture disk saves the tank against collapse. The tank contents, however, may become contaminated in the process and sanitary rupture disks are very expensive. Rupture disks outfitted with automated alarms signalling their breakage are available. Relief valves are available to redress pressure differential before closing again. They are not, however, of sanitary design, nor can they be calibrated with great precision. Water storage tanks and the conveying pipes and pumps may be sanitized by 10% (or stronger) hydrogen peroxide (H_2O_2), prepared from 30% H_2O_2. The contact time may be hours. In one such operation, the organisms count was reduced thereby from 200 to 300 cfu/100mL down to 2 to 4 cfu/100mL. Live steam may also be used to effect sanitization. In this case, the tank should be permitted to cool gradually. The tank vent must, in any case, be sized for the proper rate of air passage. Small chlorine or iodine residues are sometimes retained in storage tanks, particularly of RO water prior to mixed-bed ins tallations. Typically, 0 .2 t o 0 .4 p pm o f c hlorine o r 0 .4 t o 1. 0 p pm o f iodine residuals are utilized. Ozone concentrations of 0.5 to 1.0 ppm are very efficient as residuals [8].

CHAPTER 3

BIOLOGICAL FOULING OF HIGH PURITY WATER

Biofilms consist of cells growing in a matrix of a viscous permeable polysaccharide secretion or biopolymer and often include embedded inorganic particles such as sediments, scale deposits, or corrosion deposits. Biofilms continuously change in thickness, surface distribution, microbial populations, and chemical composition, and respond to changes in environmental factors such as water temperature, water chemistry, and surface conditions [42].

Bacteria are the most common type of micro-organism associated with the fouling of purified-water systems. Although algae, diatoms, and fungi have been isolated from such systems on rare occasions, nutritional and environmental limitations greatly restrict their growth. An understanding of the environment in which these micro-organisms grow provides a basis for selecting the appropriate testing method. It is also important to consider the mechanisms by which bacteria mediate fouling in purified-water systems [39].

A layer of living and dead organisms, their metabolic products, and various organic and organic substances trapped within a polymeric matrix of bacterial origin come to characterize virtually all surfaces in contact with water as seen in figure 3.1. As will be discussed, the formation of such a glycocalyx over time is the product of many factors. It can, however, develop rapidly, although different organisms form biofilms at different rates. Marshall (1992) cities evidence that tritium-labelled *Pseudomonas* Strain EK-20 being to form biofilm upon a polystyrene substrate within one hour. It is 75% irreversible at 6 hours, and after 22 hours is 90% so. (It should be noted, however, that the waters Marshall worked with were far more nutritious than their found in

pharmaceutical industry). Biofilms are of interest in the present context because they intermittently shed bits and pieces of their structure into the contacting waters, self-replicating entities, the shed organisms compromise the microbial integrity of the liquid; inevitably threatening thereby, the sufficiency of the WFI or Purified Water in contact with the biofilm [8].

The term "fouling" refers to the formation of inorganic and/or organic deposits on surfaces. In cooling systems, these deposits form on condenser tube walls, increasing fluid frictional resistance, accelerating corrosion and impairing heat transfer. Four types of fouling alone or in combinations, may occur:

1) Crystalline fouling caused by precitation of $CaCO_3$, $CaSO_4$ or silicates;
2) Corrosion fouling resulting from formation of insulating layers of metal oxides on the tubes;
3) Fouling due to adherence of particulate matter on tube surfaces; and
4) Biological organisms and their products, primarily extracellular polymers [58].

In production processes, which depend on the water quality, biofilms, can cause sever interferences, when micro-organisms are released from these biofilms and return to the bulk liquid phase. The production of micro-electronics, for example, is highly sensitive to particles in the water that is used to rinse the printed circuits. The source of the bacteria in the process water are:

a) Cells transported with the raw water, air, and chemicals (these cells can be filtered and are susceptible to disinfection)
b) Cells introduced by the equipment of the plant and by personnel
c) Cells released from biofilm (which are poorly susceptible to microbicides).

While abiotic particles may be controlled by sophisticated filter systems, micro-organisms are particles, which can multiply. Even very few micro-organisms, which

eventually penetrate a filter, can attach to surfaces of the water distribution system, multiply there and seed the water phase with more microorganisms that can attach to printed circuits, cause shortcuts and expensive production losses. Thus, most of the micro-organisms in high purity water systems come from biofilms rather than from breakthroughs through particle filters or filtration membranes. In pharmaceutical systems, strong efforts are necessary to avoid microbial contamination of products, even if the raw water is extensively pre-treated. It may be surprising to encounter microbial growth in high purity water, such as 18 Mohm water used in micro-electronic industry with practically no detectable organic carbon. However, micro-organisms can grow in presence of extreme low nutrient concentrations. Some starving cells are known to change their morphology and their surface characteristics, exhibiting a higher hydrophobicity and adhesiveness. Once some cells are attached to a surface in a high purity water system, they may scavenge nutrients and concentrate them locally. Considering the small size and mass of bacteria (10^{-12} g/cell) (Schlegel HG, 1985), one million cells make up a mass of 1μg, 90% of which consist of water. Thus, 10^6 cells contribute approximately 0,1μg of C_{org}, which is close to or below the detection limit of most methods. The surfaces of degasifiers frequently carry biofilms, scavenging additional nutrients from the air Geller A. (1983) showed that the nutrient uptake from the air represents a significant factor supporting microbial growth in nutrient limited systems. The inner surfaces of water pipelines can carry biofilms which increase the drag resistance, leading to more than 50% loss of transport performance, release micro-organisms to the bulk liquid, and cause episodes of elevated bacterial numbers in drinking water. Even high shear stress systems can be colonised by biofilms. Experiments with *Pseudomonas atlantica* in a shear gradient showed that a value of about 120 dynes/cm^2 represented a threshold for the attachment. A particular problem is represented by the fact that biofouling usually occurs together with other kinds of fouling (such as the deposition of minerals, organics and colloids) [31].

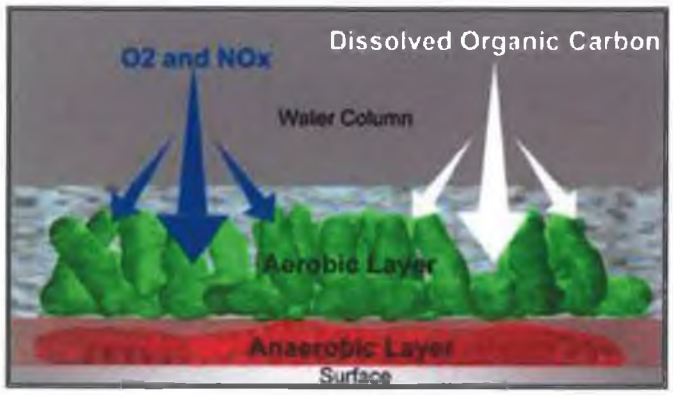


Figure 3.1. Biofouling in high purity water system

The widely-accepted model for bacterial fouling in water systems involves the following steps:

1) Attachment of organisms to a surface.
2) Absorption and metabolism of simple organic molecules that fuel further metabolic action.
3) Generation of the glycocalyx, a polysaccharide that serves to further anchor the organism and protect it from adverse environmental factors such as changes in temperature, flow, pH, or the action of biocides (above right).

Biofouling of surfaces involves living matter-either micro-or macro-organisms. The former are small, often single cell entities (e.g., bacteria, fungi, or algae) and the latter are larger creatures (such as mussels and barnacles). In heat exchangers, the problem of biofouling especially involves bacteria, although the debris from fungal and algal activity also may be a problem. In some systems, such as seawater-cooled equipment, macro-organisms may create operational difficulties. The condition is generally experienced where aqueous systems are present (e.g. in cooling water systems, paper mill operations, and in water treatment plants), but it may be present where there is a

suitable environment for microorganisms to exist and thrive. The industrial problems associated with biofouling are essentially those that accrue to all fouling mechanisms-namely, increased back pressure for a given flow rate and, in heat exchangers, reduced heat transfer for a given temperature difference [11].

The use of bacterial purified water in various industrial and medical applications has increased dramatically over the past twenty ye ars. These different applications often require varying levels of water quality. Each industry sets specifications for the acceptance of purified water quality based upon their product process demands. The aggressive nature of ionically ultrapure water (18.2 Mohm· cm) can prevent its use in, for instance, stainless steel distribution systems, which are often employed in the pharmaceutical industry. While the presence of trace levels of silica in condensate polishing loop waters creates great concern in the power industry, low-level silica contamination of purified waters used in the production of medical devices or photomasks, for example, may not constitute cause for alarm. If any one group of contaminants can be viewed as "universal" in their distribution and significance, they are the bacteria and their by-products. Their role as purified water contaminants appears to cross all boundaries of purified water application and usage. The ability of bacteria to act as self-replicating entities distinguishes these contaminants from other, abiological, particulates. Their growth, replication, and production of various ionic and organic by-products in otherwise contaminants-free purified waters presents a tremendous challenge to production and quality assurance personnel. The same metabolic and structural properties, which enable survival in such an otherwise hostile environment, create special problems for bacterial detection and treatment [25].

A wide variety of micro-organisms were detected by Devender K. Jain (1995), on the stainless steel coupon surfaces by scanning electron microscopy. Stainless steel coupons immersed in a deionised water system for about 7 months were analysed for the presence of biofouling and microbially influenced corrosion bacteria. Aerobic oligotrophs were dominant while other micro-organisms including sulfate-reducing

bacteria were present in relatively low numbers or were absent. The deionised water used in the IFB (Irradiated Fuel Bays) contained very low concentrations of organic and inorganic matter. The TOC levels in the IFB water were 0.07 ppm/l. Consequently, there was patchy growth of micro-organisms on the surface of coupons and very low microbial population was detected in the bulk phase. Ridgeway and Oslon (1982) examined the surface of pipe removed from a drinking water system, which was also oligotrophic, and noted that microbial colonisation of surfaces was significant but, in general, sparse and randomly distributed over the wall surface. Where the flow rate was very low, micro-organisms were covered with a confluent film of extracellular polysaccharide whereas in the main zone, where the flow rate was very high, micro-organisms were attached with fine strands anchored to the surface. It is unclear why there were differences in the mode of microbial attachment on the surfaces in low flow and high flow systems. It is possible that polysaccharides got sloughed off due to turbulence created by very high rates. However, both extracellular polysaccharides and fine strands can cause firm adhesion to surfaces and bind adjacent cells to initiate colony formation. Exopolymers, in addition to their role in attachment, protect cells within biofilm against phagocytosis, amoebae, bacteriophages, and endotoxins. Costerton et al. (1988) have shown repeatedly that polysaccharide polymers protect sessile microbial population from biocides. These polysaccharides act as diffusion barrier for biocides, such as antibiotics, residual chlorine and other antibacterial substances, between the bulk liquid phase and moicroorganisms embedded in the mucilage, thereby reducing their bacterial efficiency. LeChevallier et al. (1988) also showed that the age of the biofilm, bacterial encapsulation and low nutrient concentrations increased resistance of pure culture biofilms of *Klebsiella pneumonia* to chlorine. However, the most important functions of exoplysaccharides in oligotrophic systems, such as the IFB, are to capture nutrients from the bulk water and enhance transport of nutrients in the highly porous exopolymer matrix to the surface micro-organisms [15].

3.1 Surface Finish

The physical contours and the surface quality affect the overall cleanliness of the reservoir. Biofilm, the adhesion and accumulation of microbes on the wetted surfaces of the reservoir, is a chronic contamination problem. Smooth surfaces reduce biofilm formation and subsequent microbial problems. Both blow molding and rotomolding results vary with the polymer material being used. SEM images were taken of the inner surface of a polypropylene and a polyethylene storage reservoir. There was a clear difference in surface characteristics; polypropylene showed roughness and crevices, which may result in microscopic stagnation (figure 3.2) [78].

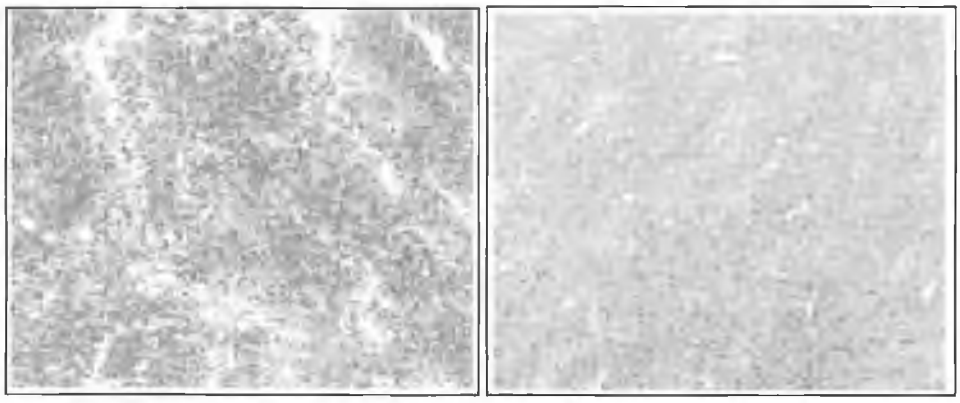


Figure 3.2. SEM images of Polypropylene (left) and Polyethylene (right) surface.

3.1.1 Abrasive Polishing: Polishing treatments bear numerical designations that imply the number of grit lines per inch; the higher the value, the smoother the surface. The final grit finish-for example, 26 should be approached progressively; first treatment uses 60 girt, then 80, and until 260 is reached. The surface should be brought down consistently from that of the raw stock. Curiously, a more reflective finish, although not so smooth a surface, may result from omitting intermediate steps. This is sometimes done to cut costs in the mistaken belief that the better-appearing surface is the smoother. The modes of

mechanical polishing can differ, as also their effect. The polishing can, for instance, be applied radially, longitudinally, or circumferentially [8].

3.1.2 Electropolishing: The use of abrasive particles to remove gross imperfections from stainless steel surfaces inevitably induces scratches, potential refuge for organisms, in those surfaces. It is said also that in mechanical polishing such as with the aid of an abrasive wheel and polishing compound, there exists a possibility of mechanically folding over or bending down of protruding metal roughness in such a way as to hide cracks and scratches and to imprison within them, for slow release, the corrosion products of the debris. This is objectionable (Artiss, 1982a). Eletropolishing, essentially the electrolytic reaction opposite to the electrodeposition of metal, is preferred. The practice employs phosphoric acid in an acidic electrolyte solution. Electropolishing is preformed after mechanical polishing. It preferential removes protruding metal, thereby smoothing roughness. It also tends to even out scratches and crevices by making them shallower and less abrupt. It thus conveys a higher degree of polish to the metal surfaces. To some small but significant dimension, iron is preferentially removed from the stainless steel surface by electropolishing. This endows the finished coating with the character of a nickel/chromium oxide cladding that exhibits enhanced resistivity to corrosion [8].

Mirror-finished electropolishing usually results after a surface has been prepared by 240-girt-or-above mechanical finishing, though some facilities apply it after a 150 or 180 finish. If a girt finish of 150, 180, or even 220 is used, however, subsequent electropolishig may still evince underlying marks of the mechanical finishing process. At least one Midwestern pharmaceutical manufacture insists on mirror-finish electropolished surfaces for parts such as pipes and tanks, and check the attainment of such quality by use of a profilometer. The present status seems to be that 316L stainless steel with mechanical finishes are widely

accepted. The FDA dose not stipulate any particular type or grade of surface finish as being required for stainless steel in contact with pharmaceutical waters, or drug or food products. No case of FDA rejection because of the finish is of common record [8].

3.2 Biofilm Formation

High-purity water literature has many articles on bacteria, sterilization, and enumeration techniques. However, with the notable exceptions of White and Mittelman, few if any of these works mention biofilms. It is believed that this stems from the "conventional wisdom" that biofilms do not exist due to the lack of nutrients and/or piping velocities that keep the pipe walls clean. Another common misconception is that biofilms may exist but do not contribute significantly to bacterial loading of the system. Loading is believed to be primary from point sources such as carbon beds, ion-exchange beds, or source water. These beliefs are false and have contributed to the continued missing of the biofilm reality in sampling programs and techniques. In a nutrient-poor system with high linear velocity and shear forces, organisms can remain in the system only if they occupy the viscous boundary layer that exists along the walls of the distribution piping. Organisms observed in the bulk liquid phase are not "resident" there, but rather reflect cells detached from the biofilm communities located on the wall [13].

Biofilms are a common form of microbial ecosystems associated with surfaces. They are found in extremely varied environments from 'pure' water systems to streams beds, ship hulls and teeth surface. In response to varying environmental conditions, biofilms develop different structures expressed in various morphologies. Many researchers have recognised the richness of morphological forms. However, characterization of biofilm morphology has been primarily restricted to qualitative descriptors such as 'smooth', 'fuzzy', and 'mushroomlike'. Recently, quantitative descriptors have been proposed including porosity and its gradients, connected porosity and fractal dimensions. These attempts are still in the developmental stage and it is likely that biofilm morphology can

be quantified by more than one parameter. Better understanding of biofilm morphology is important not only for its characterization but for description of mass transfer inside and around biofilms. While experimental methods will ultimately reveal biofilm structure, mathematical models can be useful tools for investigation of the effects of different environmental conditions on biofilm development and its morphology. Biofilm was treated as a homogeneous matrix with uniformly distributed biochemical reactive sites. A nutrient penetrated through the matrix by molecular diffusion and was transformed into products according to a prescribed local, intrinsic reaction rate. Such models were developed initially for one-dimensional geometry implying a flat biofilm. Later, two-dimensional and three-dimensional models were introduced. Additional features were further incorporated such as multiple nutrients, multiple microbial species, and variable biofilm density. A majority of existing models assumed that the biofilm composition and thickness were constant and described only nutrient transport and transformations. Some newer models included biofilm development (growth and detachment) most commonly through a biomass displacement velocity that depended on the local microbial growth rate. All these models, however, treated the biofilm as a continuum and were based on differential mass balances of various biofilm components. More important, biofilm morphology was prescribed in the models. In some models, biofilm morphology was explicitly stipulated (e.g., as a flat layer) while in other models specific assumptions were made about biofilm development (e.g., that the biomass displacement velocity is perpendicular to the substratum). In either case, the models were unable to describe complicated biofilm morphologies observed experimentally in many systems. The thickness of the boundary layer has a very strong influence on biofilm morphology. Thin boundary layers (hence low mass transfer resistance) promote the growth of dense and compact biofilms. At thicker boundary layers, much more open, dendritic biofilm forms develop, resembling 'mushroom' or 'tulips' observed in some real systems. The effect of biofilm strengths or cohesion have minor effects on its development [33].

Experimental research has shown that biofilms develop in a multitude of patterns. Traditionally, development of biofilms was seen as the formation of a layered structure growing from the substratum up. The use of one-dimensional biofilm models strengthened this view. All the property gradients (substrate concentration, biomass density, porosity, etc.) in these models are one-dimensional, varying only in the direction from the bulk liquid to the carrier surface. There is, however, significant spatial v ariability in b iofilm de nsity, p orosity, s urface s hape, m icrobial a ctivity, a nd distribution in clusters. Bishop and Rittman (1996) suggest that while one-dimensional models can be adequate for description alone, multidimensional modelling may be required for prediction of biofilm heterogeneity. In a model that predicts biofilm structural properties as surface shape, porosity, pore and channel size, these same properties are not only the output of the model but they determine also, as time elapses, the place where boundary conditions are applied. Biofilm expansion is mainly due to bacterial growth and production of extracellular polymers. Nutrients necessary for bacterial growth are dissolved in the liquid flow and reach the cells by passing first through the mass transfer-boundary layer (external mass transfer) and then through the biofilm matrix (internal mass transfer). The external mass transfer resistance is given by the thickness of the Concentration Boundary Layer (CBL), which is directly correlated to the Hydrodynamic Boundary Layer (HBL) resulting from the flow pattern over the biofilm surface. On one hand, the fluid flow drives the biofilm growth by regulating the concentration of substrates and products at the liquid-solid interface. On the other hand, the flow shears the biofilm surface, eroding the biofilm structural protuberances. While the flow changes the biofilm surface, the interaction is reciprocal because a new biofilm shape leads to a different place of boundary condition, thus different flow and concentration fields. This further leads to the concept of temporal heterogeneity: The biofilm is a dynamic structure evolving in non-steady-state conditions. For a bofilm model capable of full description of the three-dimensional heterogeneity, it is of importance that the model can easily cope with a large variation in time constants and with a continuously changing liquid-biofilm irregular boundary. With this in mind, Picioreanu Cristian (2000) started to develop a quantitative model based on a discrete

algorithm. Similar approaches have been independently reported by Wimpenny and Colasanti (1997) and Hermanowicz (1998, 1999). Although these later models generate biofilms with qualitative features somehow resembling observed biofilms, these models work in a completely abstract time and space. There is no direct link between model parameters and real values of some widely accepted parameters as diffusivities, reaction constant, etc. Moreover, it have been found that of some of the processes a traditional differential approach is not only computationally but also conceptually more advantageous, which led to a combined discrete-differential model for the formation of biofilms. Besides diffusion, convection is important for the overall mass transport toward the biofilm. Experimental by de Beer and Stoodley (1995) have shown that the concentration boundary layer can be parallel to the substratum (at low flow velocity) but can also follow the biofilm shape (at higher flow velocity). Modelling fluid flow will also eventually give the possibility of modelling detachment due to liquid shear stress. Recently, Picioreanu Cristian et al. (2000) described the influence of convective transport on substrate conversion in geometrically heterogeneous biofilm structures. A first effort to explain biofilm heterogeneity with a 2-D model including hydrodynamic processes, substrate transport by diffusion and convection, biomass growth and spreading is reported in Piciorenu et al., (1999), in his study (2000) the model is used to systematically investigate the role of fluid flow, internal and external nutrient mass transport and inoculums distribution on development of the biofilm structure, biomass growth and biomass spreading. It was found that in the absence of detachment, biofilm heterogeneity is mainly determined by internal mass transfer rate of substrates and by the initial percentage of carrier-surface colonization. Model predications show that biofilm structures with highly irregular surfaces develop in the mass transfer-limited regime. As the nutrient availability increases, there is a gradual shift toward compact and smooth biofilm. A smaller fraction of colonised carrier surface leads to a patchy biofilm. Biofilm surface irregularity and deep vertical channel are, in this case, caused by the inability of the colonies to spread over the whole substratum surface. The maximum substrate flux to the biofilm was greatly influenced by both internal and external mass transfer rates, but not affected by the inoculation density [68].

3.3 The Aim Of The Study

The industrial importance of biofilm development in tubular flow systems is unquestionable. Corrosion acceleration, decreasing flow capacity and product quality in transport systems is affected by microbial biofilms. Energy losses due to fouling cause significant economic impact in heat exchanger equipment. Health infection problems are have been caused by biofouling. The phenomenon of bacterial adherence to surfaces is of general interest to microbiologists and the nature of sessile populations in engineered tubular flow systems is, therefore, an active area of research. The development and attachment of films on surfaces are complex phenomena and depends upon conditions affected growth and reproduction. The levels of fluid v elocities are influence the shearing action and fractional factor.

It is therefore of interest to investigate the effect of fluid velocity levels on the development of biofilms under carefully controlled conditions.

The objectives of this study can be summarised as follows:

- Development of a rig suitable to the formation of biofilm.
- Development of a method of easy immersion and removal of samples from the rig for inspection.
- Detection of biofilm using a simple staining method.
- Investigate the effect of water flow velocities in the biofouling sedimentation.
- Use of Computational Fluid Dynamics (CFD) to analysis fouling problems.

CHAPTER 4

RIG DESIGN AND EXPERIMENTAL SET-UP

4.1 RIG DESIGN

To investigate fouling in a high purity water system a pump rig and reservoir was modified to allow easy access to sample slides.

The centrifugal water pump was driven by an electric motor (figure 4.1), which was mounted on a support plinth together with a clear acrylic reservoir and associated pipework for continuous circulation. High purity water was used as the operating fluid and a drain valve at the base of the reservoir allowed the water to be drained after use. Appropriate sensors were incorporated on the unit to facilitate analysis of the pump performance when connected to the parallel port of a suitable microcomputer via an Armfield "POD" interface. In addition to the tappings required by the pressure sensors, additional tappings were included in the pipework to allow appropriate calibration instruments to be connected.

The flow of water through the centrifugal pump was regulated by a flow control valve installed in the discharge pipework of the pump. Adjustment of this valve allows the head / flow produced by the pump to be varied.

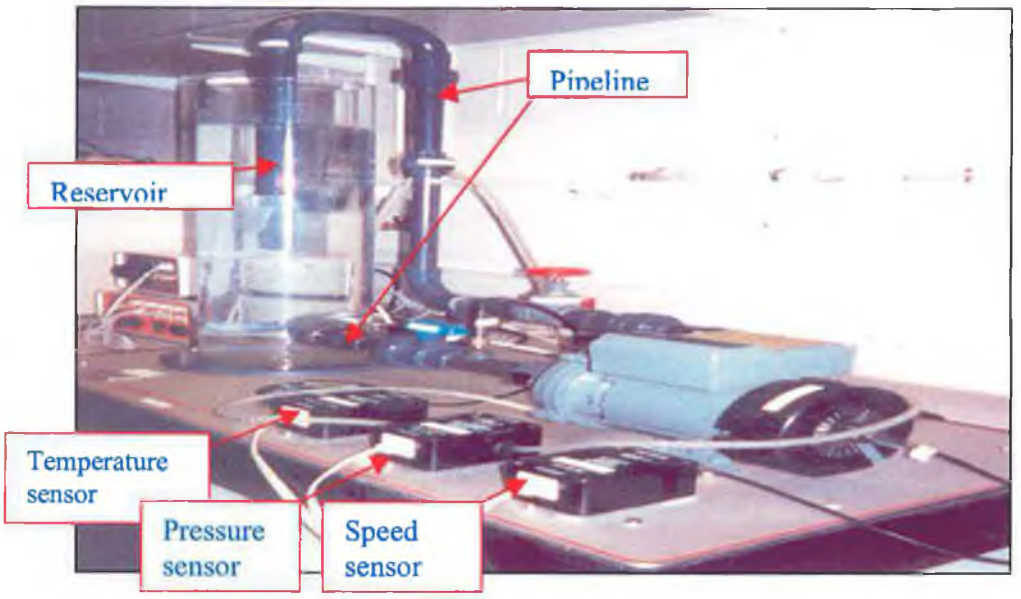


Figure 4.1. Centrifugal Pump Demonstration Unit (FM20)

The following sensors were used to monitor the performance of the system:

4.1.1 Differential pressure sensors SPW1

This device was used to measure pressure developed across the orifice plate installed in the discharge pipework of the pump. The volume flow rate of water through the pump can be calculated using this measurement. The sensor is connected to the appropriate tappings in the pipework using flexible tubing. Additional tappings are provided for the connection of appropriate instrumentation to facilitate calibration of the differential pressure sensor.

4.1.2 Differential pressure sensor SPW2

This comprises of a pressure sensitive piezoresistive device with appropriate signal conditioning all contained in a protective case and is used to measure the difference in pressure between the inlet and outlet of the centrifugal pump. The head developed by the pump can be calculated from this measurement. The sensor is connected to the appropriate tappings in the pipework using flexible tubing.

4.1.3 Temperature Sensor

This comprises of a temperature sensitive semiconductor device on a remote lead with appropriate signal conditioning in a protective case and was used to measure the temperature of water entering the centrifugal pump. The sensor is inserted through the wall of the pipe using a waterproof gland.

In addition to the above sensors, a watt-meter was connected to the rig. The Wattmeter was connected between the main lead from the pump and a suitable power supply to facilitate measurement of the electrical power supplied to the motor. The Integrating Wattmeter may be calibrated using a suitable twin trace oscilloscope.

4.2 Operation of the Rig

The inlet valve (9) was opened and the outlet control valve (16) closed. Ensure that all the drain valve (10) at the base of the reservoir is fully closed then fill the reservoir with clean, cold high purity water. The pressure sensors on the unit require priming with water before initial operation (and whenever the tank has been emptied and refilled). A hypodermic syringe and micro-bore tubing was used for this purpose.

Remove the flexible tubing from the PVC pipe, to prime the tube with water, by removing the pipe clip and gently pulling the tube from the stainless steel tapping. The syringe was filled with water and gently insert the micro-bore tubing into the sensor's flexible tubing until it is a few millimetres away from the sensor. The flexible tubing was held vertically. Slowly water was inserted into the tube until it is completely filled, then removed syringe and micro-bore tubing, and replace the flexible tubing on to the stainless steel tapping. Two stainless steel tappings were located at each tapping point. One connected to the sensor, whilst the other is used to connect a manometer. The flow control valve fully was opened and water circulated until all air bubbles are expelled. Switch off the unit (Rig). Each of the sensors were connected to conditioning boxes to the appropriate sensor sockets on the front of the unit.

4.3 Pressure measurement comparison between the Manometer-H30 and centrifugal pump demonstration unit-FM20

To ensure of the accuracy of the rig pressure measurement, a comparison of the pressure measurement was made between the rig pressure sensor and a calibrated manometer.
The inclined U tube manometer was connected to FM20 pressure valves at the pressure points P4 and P6. The pressure readings were taken in different fluid velocities (pump speed) and three different positions of flow control valve (value 1 turn, value 2 turn and value full open) and changes to the connection of pressures sensor on the pressure tappings of the Rig as seen below:
Step (1): The pressure sensor connected to P1+P3, P5+P6.
Step (2): Ignore connection of pressure sensor to P1+P2 and P3+P4, while connected to P5+P6.
Step (3): The pressure sensor connected to P2+P3, P5+P6.
See Figure (4.2) for details.

4.4 Modifying FM20 unit to include slides

The rig was modified to include some additional pipe work and small pieces of glass slides.
New horizontal pipe work (has U shape, as seen in figures 4.3, 4.7, and 4.8) of the same pipes material, PVC, and the same diameter (22mm diameter) was installed in the pipe work of the Rig in the position 12 (Figure 4.1) instead of the junction which join the last two vertical pipes before entering the tank (as seen in figures 4.2 and 4.3). A specially designed glass slide 19mm wide, 25mm long and 1mm thick was inserted in the modified pipework to analyse biofilm formation.

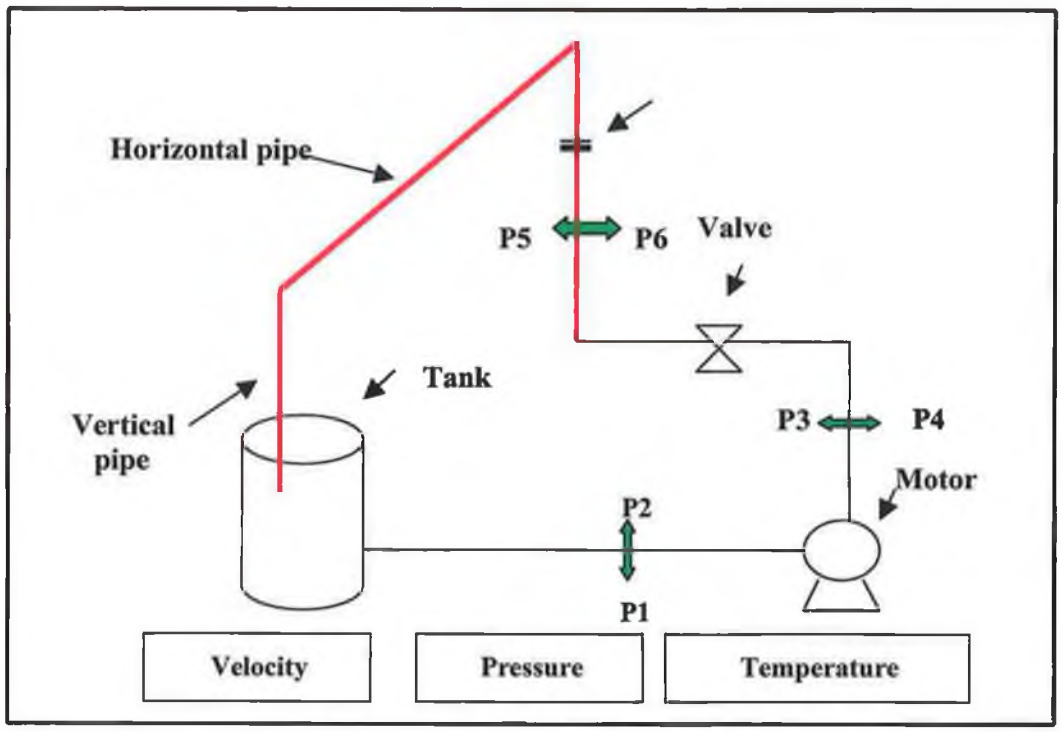


Figure 4.2. The Rig before additional pipe work
(P1, P2, P3, P4, P5 and P6 = pressure sensors on the Rig)

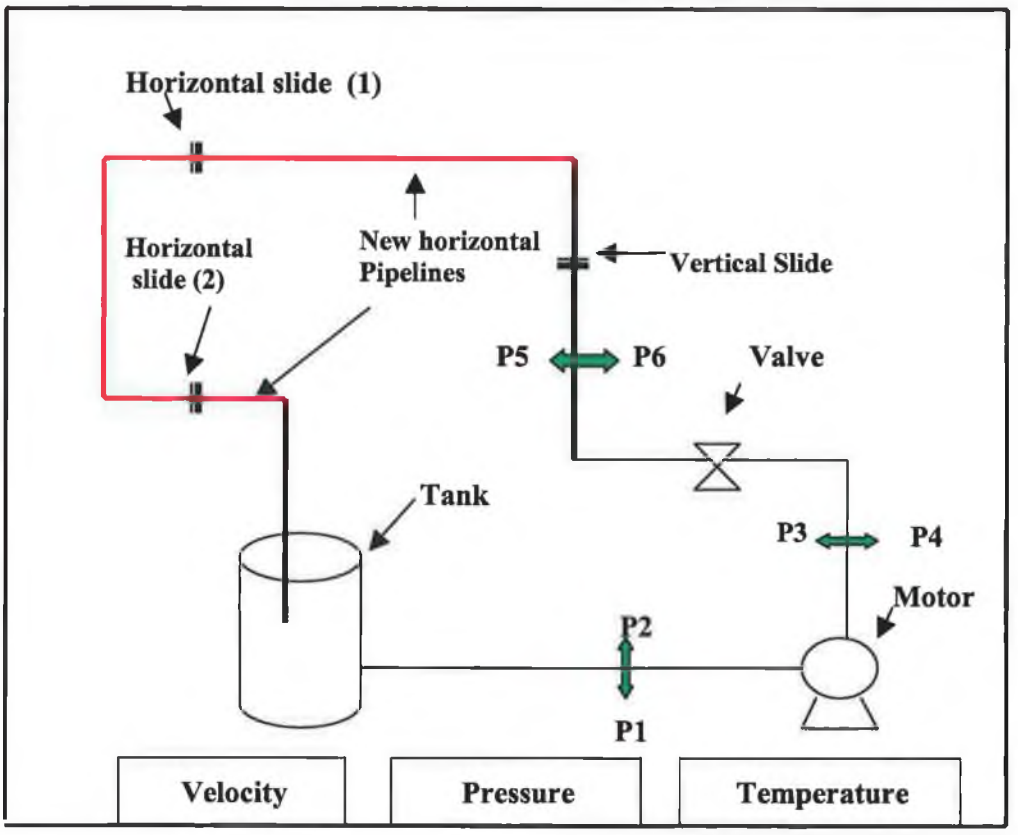


Figure 4.3.The modification of the Rig (addition U tubes)

4.5 The Rig Before modification

Figure 4.4. The vertical and horizontal tubes

Figure 4.5. The Rig (FM20)

Figure 4.6. The pressure and temperature sensors of the Rig

4.6 The Rig after modification

Figure 4.7. The modified horizontal tube

Figure 4.8. The vertical and horizontal tubes

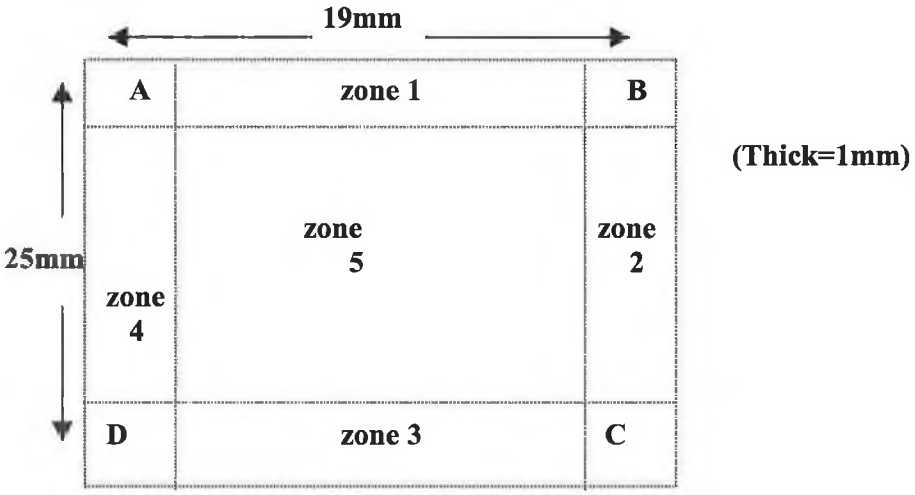


Figure 4.9. Sketch of the slide

4.7 The slide location

The slide is the same type used for the light-microscopic tests (figure 4.9). These long slides were sheared into small piece of 19mm length and 25mm width and thick (1mm thick). Five zones were highlighted on the slide to facilitate the counts of the cells and investigate the position on the slide. Zone (1) for the front (flow in), zones (2 and 3) for the sides, zone (3) for the rear (flow out), and zone (5) for the centre of the slide. A, B, C and D represent the corners of the slide. These zones apart from the centre of the slide had an area of approximately $5mm^2$.

4.8 The Experimental Method

- The covered reservoir was filled with high pure water and the rig run for several days with out change into the water to allow the Rig to be contaminated by microorganisms.
- The front and rear sides (side 1=flow in, side 3=flow out) were detected on clean and sterile slides before being inserted carefully into the vertical and horizontal pipes at the position (12) (figure 4.1). The pipes and the joints were closed tightly.
- The Rig was operate at specific motor speeds (30,40,50,60,70 and 80) for several minutes before take the reading and the measurement from the computer to ensure no air bubbles were in the tubes and the water velocity and the pressure monitored from the computer. Flow velocities of 0.0081,0.0776,0.1332 and 0.1598 m/s were tested. The flow velocities were equivalent to the following Reynolds numbers: Re of 178.2, 1707.2, 2930.4 and 3515.6.
- The Rig was operated for 24 hours at specific motor speed and flow velocity.

4.9 Laboratory Experiment

- After 24-hour operation at a specific motor speed and flow velocity, the vertical and horizontal pipes were opened and slides removed very carefully.
  The slides were placed on a dry clean tissue in a closed dry place for 24 hours to dry and fix the microorganisms on the slides before staining.
- Gram's stain was used to stain the slides, (the steps of the stain is explained below) and the slides then left to dry for a few hours before examining for the presence and accumulation of the microorganisms.
- The presence and accumulation of the microorganisms on the slides was tested under the light microscope in nine different positions on the slides (1= front side, 3=rear side, 4,2= left and right hand sides, 5=the centre, A, B, C and D the corners), Figure (4.9).
- The number of the microorganisms in each zone on the slide was counted separately and then accumulated to give a total count for every slide for specific motor speed and flow velocity for vertical and horizontal positions (tables7.1, 7.2 and 7.3).
- The counts and the accumulation of the microorganisms were compared with the effect of water velocities, Reynolds number and the pressure.

4.10 The Gram Stain Method

1) Purple dye crystal violet was used as a primary stain.
2) Iodine was then applied, which acts as a mordant.
3) A decolourising agent, an acetone solution was used. At this stage the gram negative bacteria lose their violet stain, but gram-positive bacteria do not.
4) The pink dye safranin is added as a counter stain. Its turns the decolorized gram negatives pink and the gram-positive bacteria a deeper violet colors. This allowed the author to distinguish between each type of bacteria and to easily count the number of cells accumulated.

Chapter 5

5.1 THE LABORATORY EXPERIMENTAL RESULTS

Following staining of the horizontal and vertical slides, each slide was examined for the presence of bacteria. Filamentous bacterial cells were observed in each run and it was found to be the dominant of all cells. Generally, the front of the slide (zone number 1,or the flow inlet side) had the highest number of cells, more than the other zones of the slide, and more cells were on the horizontal slides than the vertical slide (tables 5.1,5.2, and 5.3). The back of the slides (flow outlet side) cells were less evident. A/ B (front) location of slides relative to pipe is important, the cells concentrated on these corners more than the others (C, D)(back). The centre of the slides had the lowerest number of built up cells. Also, the sides of the slides (no. 1 and 2) had high counts. The counts of the microorganisme in the horizontal slides are higher than in the vertical slides.

The number of the cells deposited was affected by the velocity of the flow and the Reynolds number. As the water flow velocity is increased the number of cells deposited increased.

The motor speeds of 40,50 and 60 Rpm (Laminar velocity of 0.0081 m/s and Re number of 178.2) gave the lowest cell counts, and these increased gradually with increases in the motor speed of 70 (water velocity of 0.1332 m/s and RE number of 2930.4) and with motor speed of 80 (water velocity of 0.1598 m/s and RE number of 3515.6).

The horizontal slide, zone (2), the cells counts were fluctuating. The cell counts were low at a motor speed of 40 (water velocity of 0.0081 m/s and RE number of 178.2), and then increased sharply with motor speed of 50, which still is in the laminar flow. In laminar velocity of motor speed of 60 it decreased again to approximately the half-cell

counts then increased again gradually in the turbulent flow of motor speed of 70 (water velocity of 0.1332 and Re number of 2930.4) and motor speed of 80 (water velocity of 0.1598 and RE number of 3515.6).

The comparison between motor speed (Fluid velocity) and cells counts is given in tables (5.1 and 5.2), and indicates clearly these results. The cells counts increased with increasing turbulent flow. The Figure 5.6 show how results fluctuated; the highest counts were in velocities of motor speed of 60 that is laminar, then at motor speed of 80, which is turbulent.

Rig. Velocity m/s	1	2	3	4	5	Total
0.0081	90	104	27	19	7	**247**
0.04	11	30	36	12	5	**94**
0.0776	23	10	8	3	8	**52**
0.1332	42	62	26	147	37	**341**
0.1598	88	41	287	25	50	**491**

Table 5.1. Cells counts in the vertical slides

Rig. Velocity m/s	1	2	3	4	5	Total
0.0081	9	14	13	114	54	**204**
0.04	5	12	3	0	0	**20**
0.0776	156	151	117	33	67	**524**
0.1332	142	1500	200	18	90	**1960**
0.1598	500	360	55	92	158	**1165**

(±) Medium cells count, (+) high cells count, and (-) negative cells count

Table 5.2. Cells counts in the Horizontal slides (1)

Rig. Velocity m/s	1	2	3	4	5	Total
0.0081	26	30	6	5	0	**67**
0.04	1000	2	6	0	6	**1014**
0.0776	118	30	141	12	174	**475**
0.1332	151	75	4	125	27	**382**
0.1598	438	141	300	40	55	**974**

Table 5.3. Cells counts in the Horizontal slides (2)

Rig. Velocity m/s	Total Biofouling			
	Vertical	Horizontal		Cell total
0.0081	247	204	67	**518**
0.04	94	20	1014	**1128**
0.0776	52	524	475	**1051**
0.1332	341	1960	382	**2683**
0.1598	491	1165	974	**2630**

Table 5.4.Total biofouling counts on the slides.

Motor speed	Water flow m^3/s	Velocity m/s	Vel. Average	RE	RE Average	Laminar Turbulent
40	0.000031	0.0081		**178.2**		**Laminar**
50	0.000031	0.04		**816**		**Laminar**
60	0.000293	0.076	**0.0776**	1672	**1707.2**	**Turbulent**
70	0.000527	0.138	**0.1332**	3036	**2930.4**	**Turbulent**
80	0.000593	0.156	**0.1598**	3432	**3515.6**	**Turbulent**

Table 5.5. The average of the water velocities and Reynolds numbers.

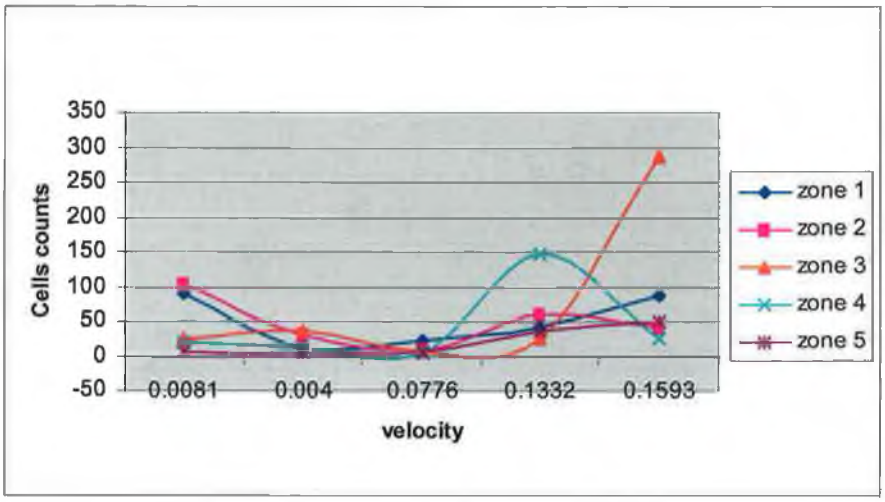


Figure 5.1. Cell counts on vertical slide

- Back of the slide had highest counts at high velocity.
- All zones decreases with increasing velocity up to 0.0776 m/s, then all steadily increased.
- Wall regions show higher counts up to 0.1332.
- Slouching on removal.

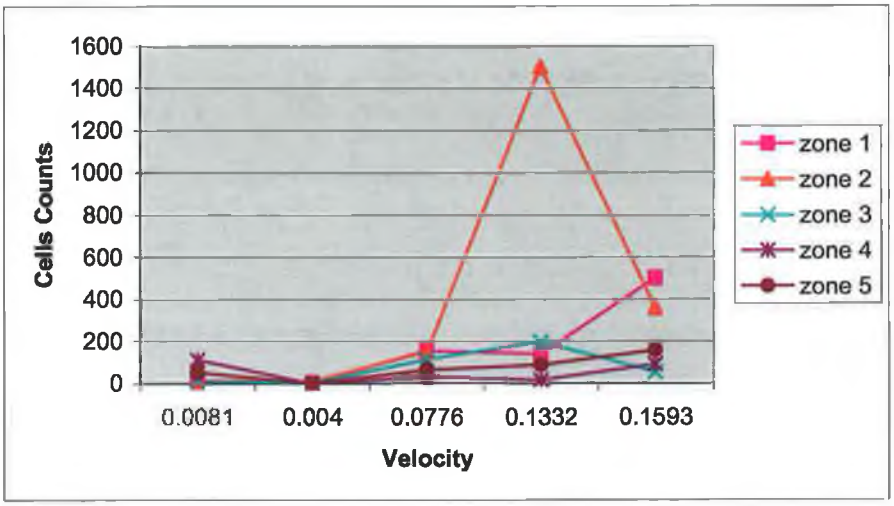


Figure 5.2. Cell counts on Horizontal slide (1)

- Entry length may have made a different result.
- Large increase in counts from 0.0776 m/s.
- Front of slide increased with increasing velocity.
- Zone 2-wall region may have experienced low flow at high velocities.

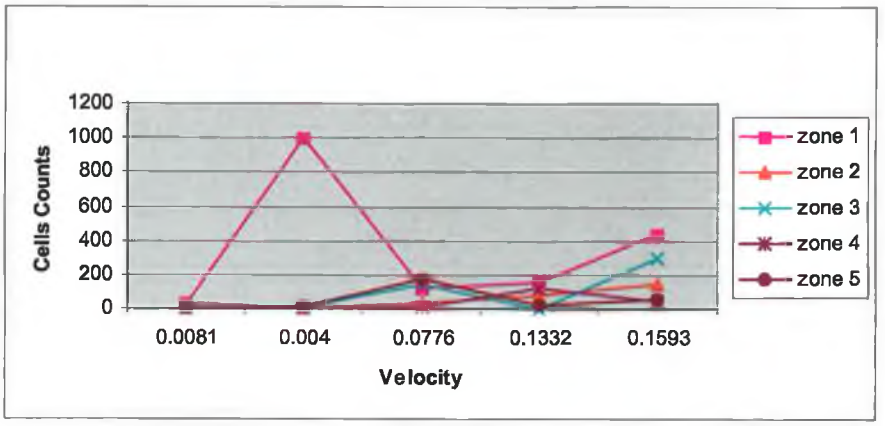


Figure 5.3. Cell count on horizontal slide (2)

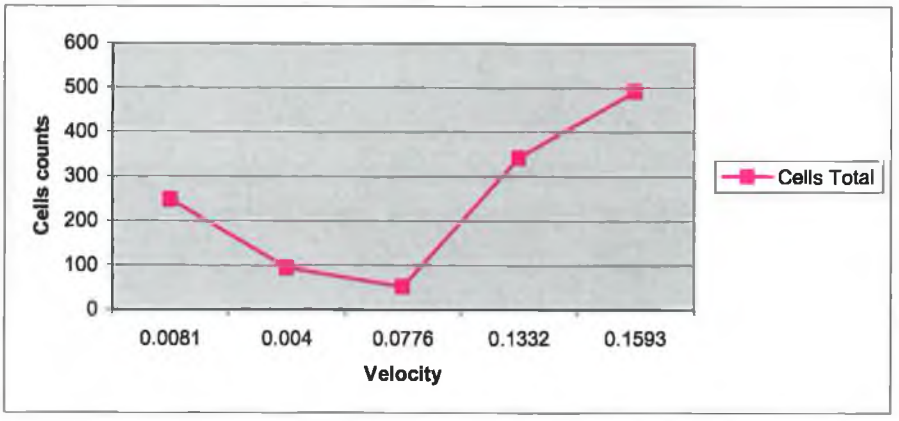


Figure 5.4. Total cell count on vertical slide

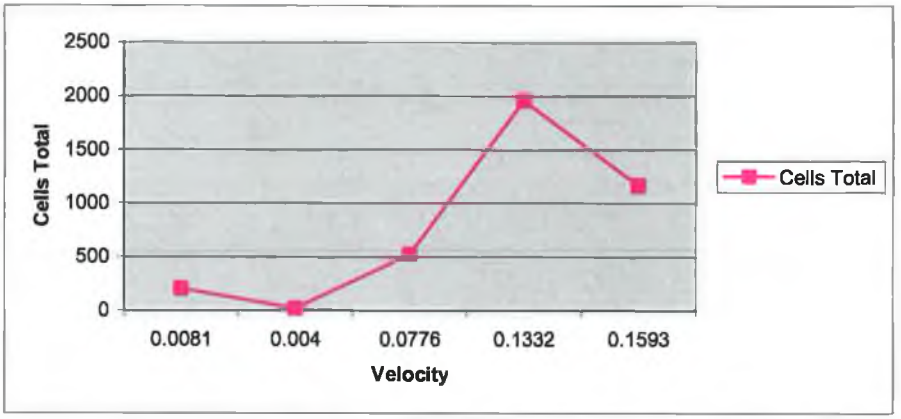


Figure 5.5. Total cell counts on horizontal slide (1)

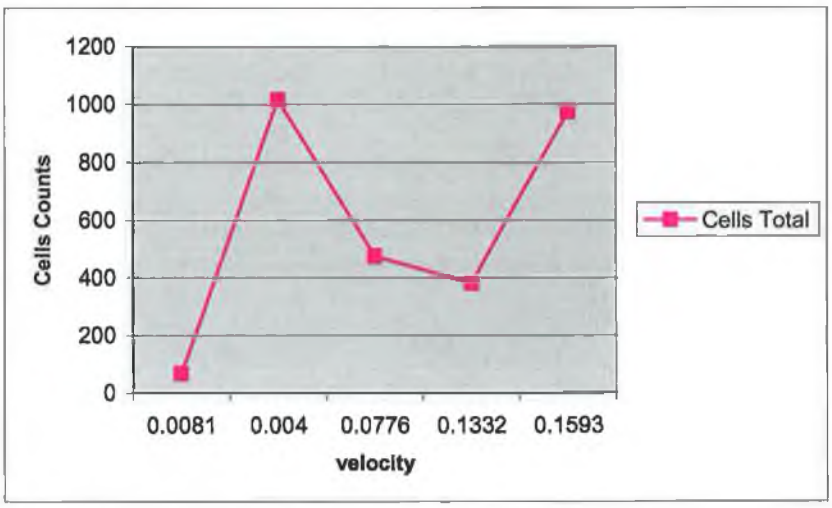


Figure 5.6. Total cell count on velocity horizontal slide (2)

- Lower counts on slide 2 may be due to exit effect and separation due to the bend in the pipework immediately before the slide, i.e. no extry length exists before this slides.
- The horizontal slides show higher counts than the vertical and the total cell counts in figure (5.5) show a total increase in bacteria with increased velocity.

CHAPTER 6

COMPUTATIONAL FLUID DYNAMICS ANALYSIS AND RESULT

Gambit is an integrated pre-processor package for CFD (Computational Fluid Dynamics) analysis. The package allows geometry to be constructed using bottom-up or top-down techniques or may be imported from an alternate package. Its capabilities include:

- ACIS solid modelling capabilities.
- IGES import, cleanup and modification.

Gambit allows the construction and meshing of models by means of its graphical user interface. It is used to generate meshes for all Fluents solvers and it offers a wide range of elements and schemes including structured and unstructured hexahedral, tetrahedral, pyramid and prisms. Once generated the mesh quality may be analysed and modified if necessary. The general sequence of operations for geometry construction and meshing is as follows:

1. ***Initial set-up:*** This includes solver selection; mesh size specification and defaults settings.
2. ***Geometry creation:*** Full geometry creation or decomposition into meshable sections.
3. ***Meshing:*** Edge and boundary local meshing or face and volume global meshing.
4. ***Mesh Examination:*** Mesh quality analysis and modification.
5. Zone assignment and mesh export.

6.1 The Procedure For Meshing The Model

1. The Fluent 5/6 solver was chosen to run CFD calculation.
2. Both geometries were created, first for the large pipe of 22mm diameter and 219 long. Second geometry for small slide inside the pipe of 19mm length and 1mm thick, (figure 6.1).
3. The faces were created for the large pipe and the small slide to align the forms.
4. Subtract real faces to perform a Boolean subtraction involving two real faces.
5. Specify the edges of the faces for the meshing operation by use of the reserve option and double-sided grading. The Ratio geometry was 1.0.
6. Specify the node distribution on the edges of both faces to define the grid density on the edges of the geometry, assign the number of nodes, and specify the distribution of nodes along the edge. Select the Interval count option (The interval length ratio, R, is a function of both the edge length, L, and the number of intervals). The spacing geometry was 1.0.
7. A structured mesh was created on the faces (figure 6.2) by using 2D class-QUAD shape-element (option -high quality hex mesh) in order to reduce discritisation errors. Then the Map option was specified to create a regular, structured grid of mesh elements. When apply the Quad-Map meshing scheme meshes the face using a regular grid of quadrilateral face mesh elements, as seen in figures (8.4 and 8.5). Meshing geometry spacing used was 1.0.
8. The Boundary types (the inflow and the outflow the pipe) were used to define the spacing of mesh node rows in regions immediately adjacent to edges and/or faces. They are used primarily to control mesh density and, thereby, to control the amount of information available from the computational model in specific regions of interest. Cell density of 7,500 cells.
9. The mesh was then exported and the session saved as a case file.

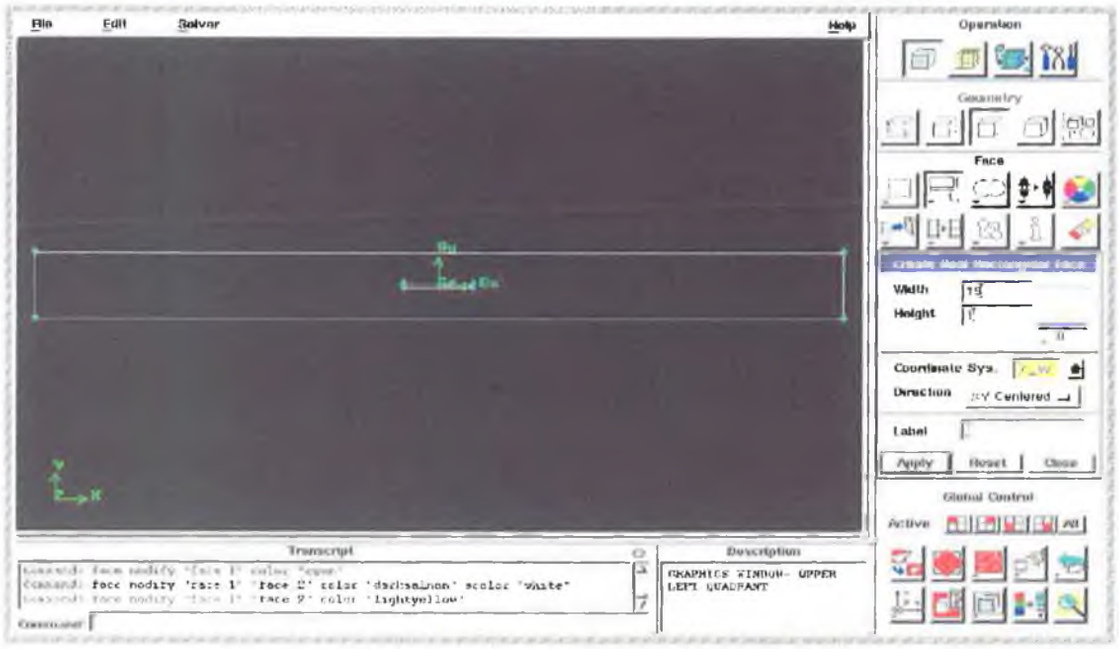


Figure 6.1 Geometry creations for the pipe and the slide

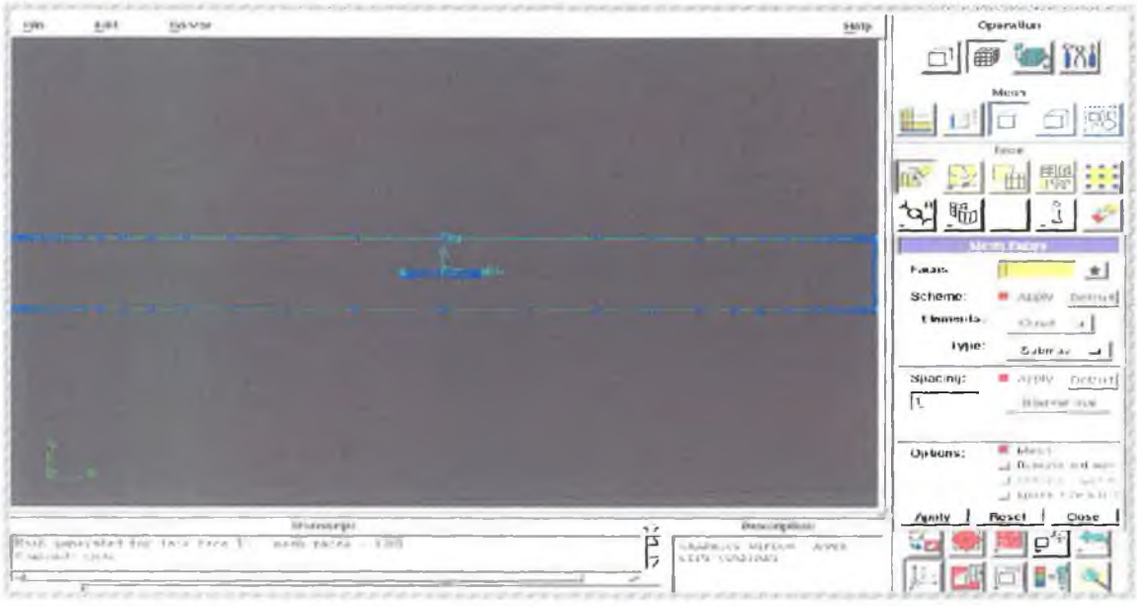


Figure 6.2.Specification of the node distribution

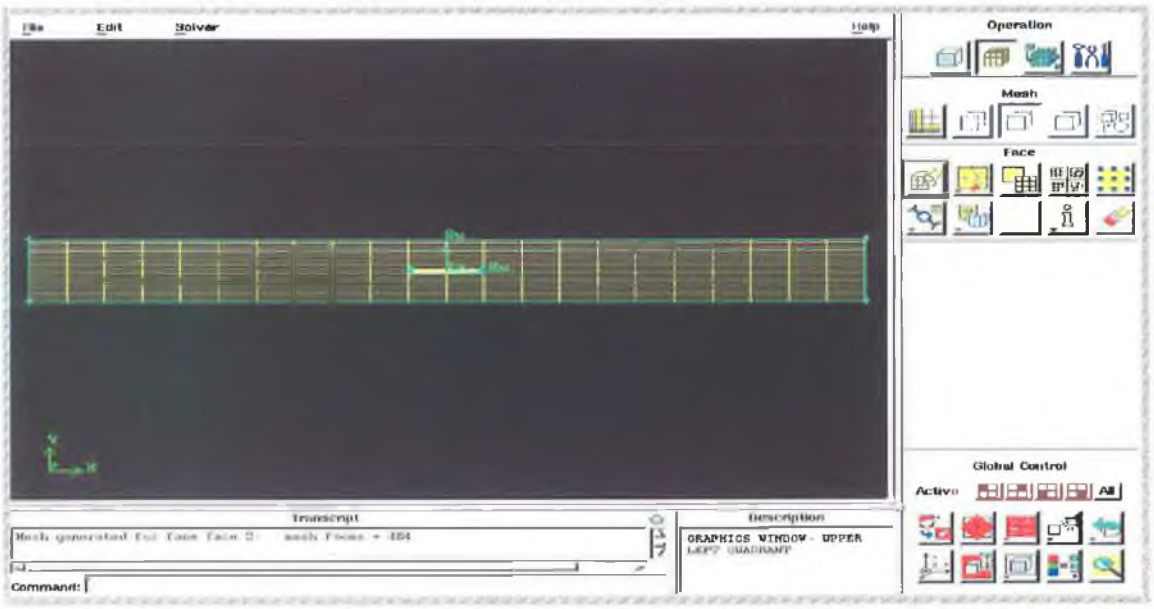


Figure 6.3.The final structured mesh

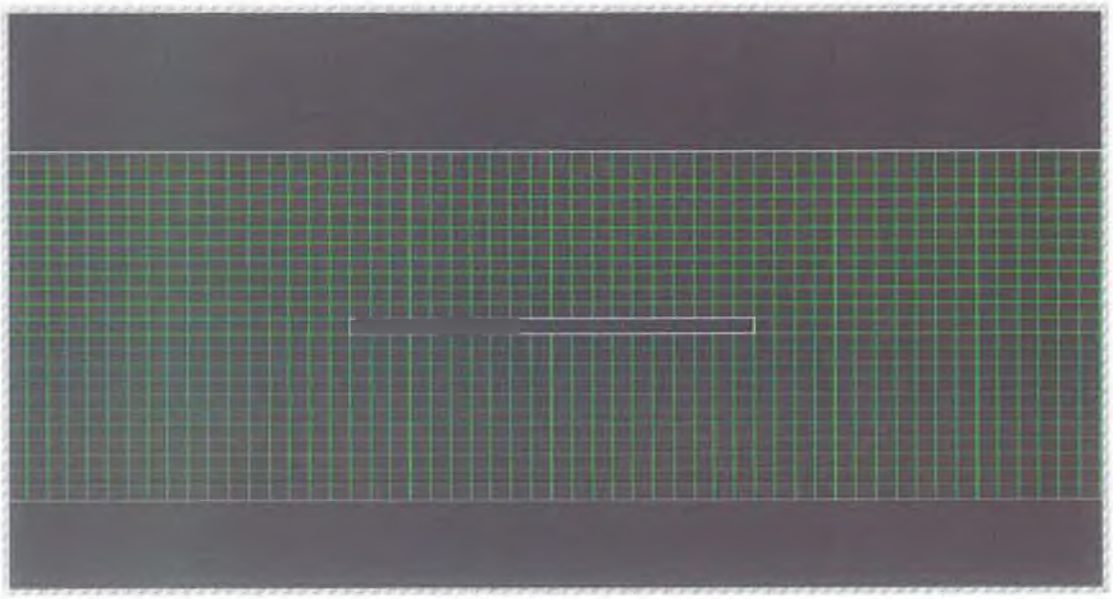


Figure 6.4. Exploded view of the slide and tube mesh

6.2 Fluent solver

The current version of Fluent 6.1 offers use of three numerical methods when solving flow problems, the segregate solver method or the coupled implicit method. The segregated solver method will solve continuity, momentum, and (where appropriate) energy and species equations sequentially (i.e., segregated from one another). These formulations solve the equations for additional scalars (e.g., turbulence or radiation quantities) sequentially and is preferred as it has lower memory requirements and provides flexibility in solution procedures. Using either method Fluent (segregated method or coupled method) will solve the governing integral equations for conservation of mass and momentum and when appropriate energy. The segregated solve was used.

6.3 Set up of model in fluent

In this thesis we need to analyse two kinds of fluid velocities flow and there effect on the fouling sedimentations inside the pipe and especially around the small slide inside the pipe. Laminar and turbulent. Therefore we used two models for the analysis:

1. Laminar model for the laminar flow velocity.
2. The Standard k- ϵ model used in this thesis for the turbulent flow velocity. It's the simplest ``complete model" of turbulence are two-equation models in which the solution of two separate transport equations allows the turbulent velocity and length scales to be independently determined. The standard k- ϵmodel was used for all studies.

Robustness, economy, and reasonable accuracy for a wide range of turbulent flows explain its popularity in industrial flow and heat transfer simulations. It is a semi-empirical model, and has achieved considerable success in modelling a wide variety of flows without the need for case-by-case adjustment of the model constants.

6.4 Material definition

An important step in the set-up of the model is the definition of the material and this material is then assigned as boundary conditions for zones. In this problem the fluid was water.

6.5 Defining Boundary Conditions

Boundary conditions specify the flow variables on the boundaries of the physical model. They are, therefore, a critical component of our fluent simulations and it is important that they are specified appropriately. Boundary conditions were applied to the inlet, outlet and the wall of the pipe. The boundary types used in this model were:

- Flow inlet and exit boundaries: velocity inlet, outflow.
- Wall boundary conditions.
- Internal cell zones: fluid (water).

6.6 Plots of data

To detect the results in the domain, a series of lines were created in the tube. A line surface is simply a line that includes the specified endpoints and extends through the domain; data points will be at the centres of the cells through which the line passes, and consequently will not be equally spaced.

Line location	Position
X line – 100	inlet flow
X line –60	60 mm upstream
X line – 35	35 mm upstream
X line – 25	25 mm upstream
X line – 15	15 mm upstream
X line – 10	10 mm upstream
X line – 5	5 mm upstream
XY line 0	**The centre of the tube**
Y line + 5	+5 mm downstream
Y line + 10	+ 10 mm downstream
Y line + 15	+ 15 mm downstream
Y line + 25	+ 25 mm downstream
Y line + 35	+ 35 mm downstream
Y line + 60	+ 60 mm downstream

Table 6.1.Evaluation position within the domain.

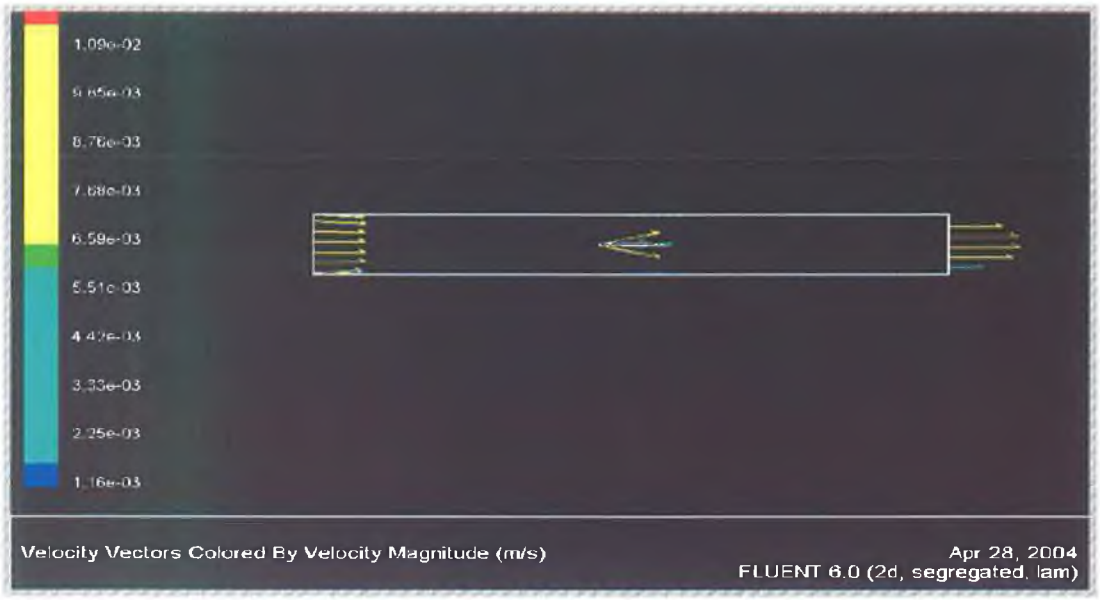


Figure 6.5. The boundary conditions

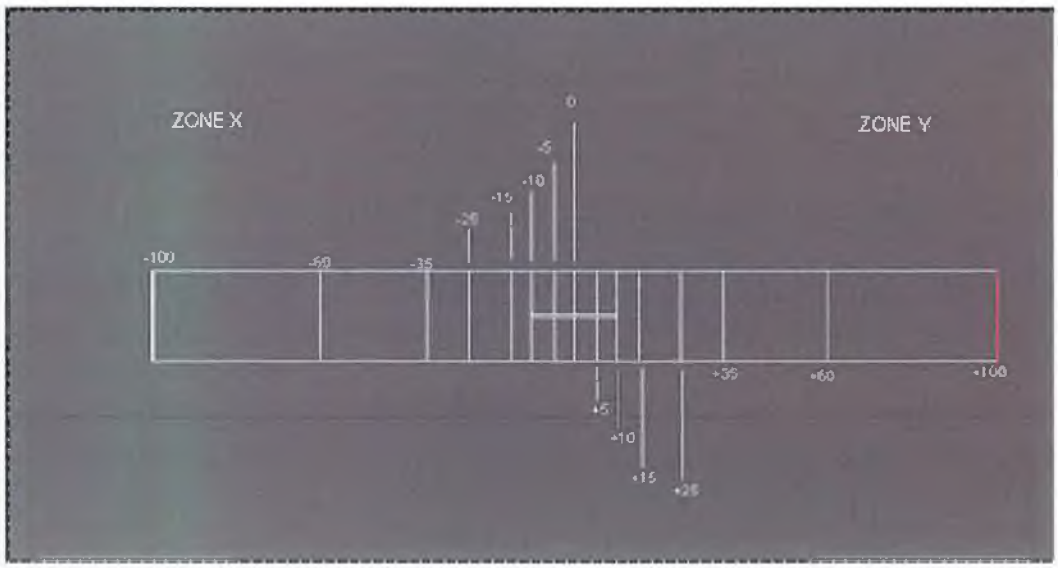


Figure 6.6. Plots position on the tube

CHAPTER 6

THE FLUENT RESULT

6.7 Laminar velocity 0.0081m/s

Reynolds number of 178.2
(Low Velocity Run)

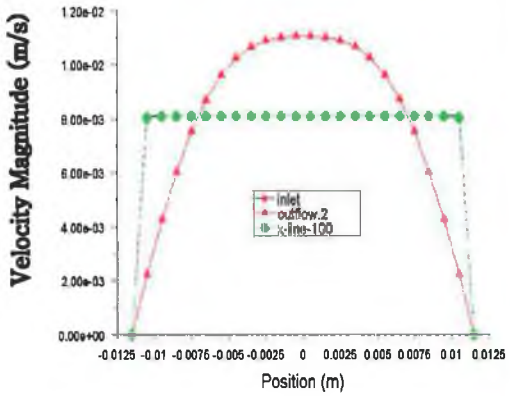


Figure 6.7.Velocity profile at plot –100

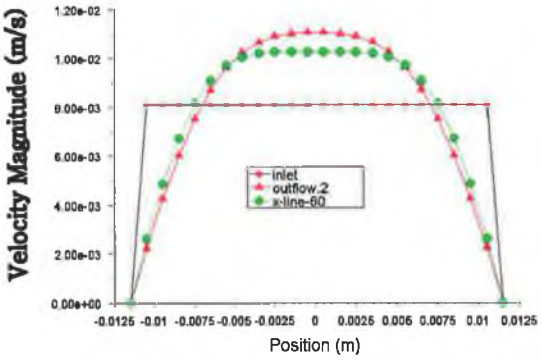


Figure 6.8. Velocity profile at plot -60

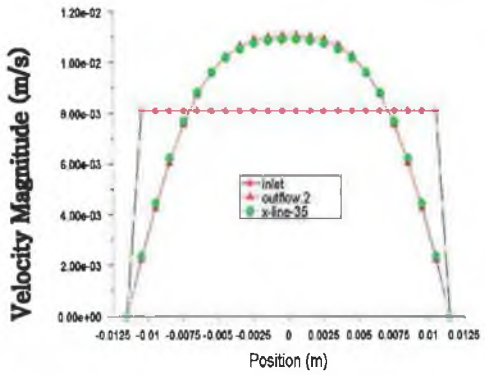


Figure 6.9. Velocity profile at plot -35

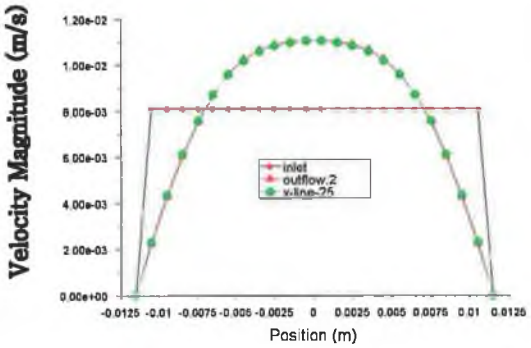


Figure 6.10. Velocity profile at plot -25

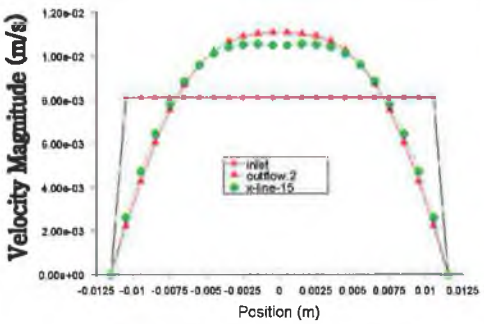


Figure 6.11.Velocity profile at plot –15

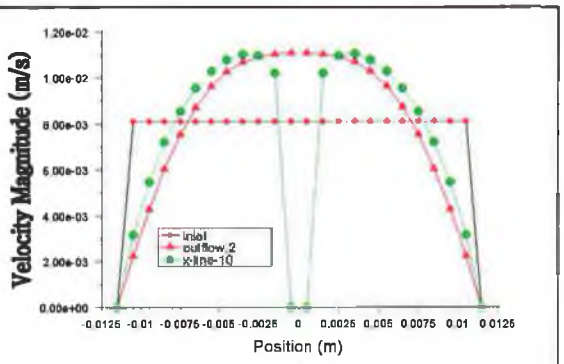


Figure 6.12.Velocity profile at plot -10

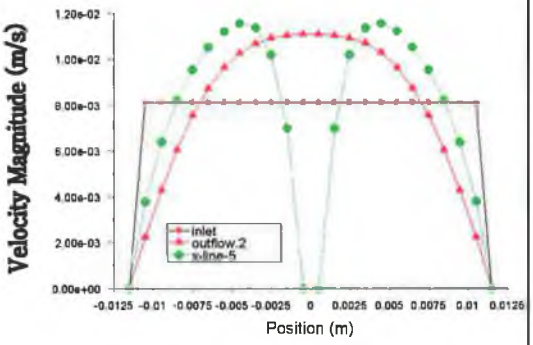


Figure 6.13.Velocity profile at -5

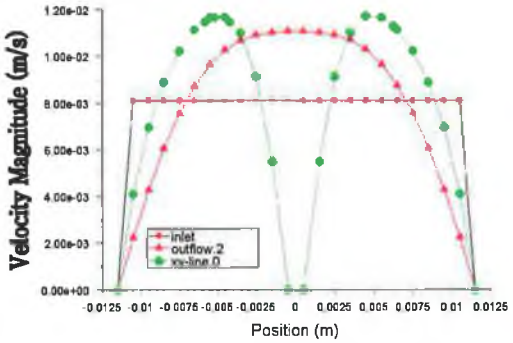


Figure 6.14.Velocity profile at plot zero

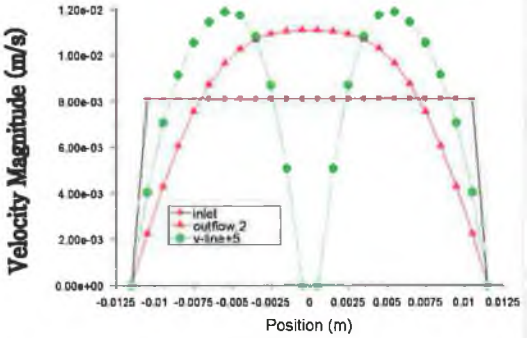


Figure 6.15.Velocity profile at plot +5

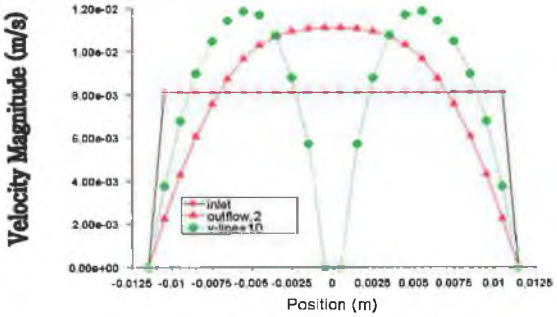


Figure 6.16. Velocity profile at plot+10

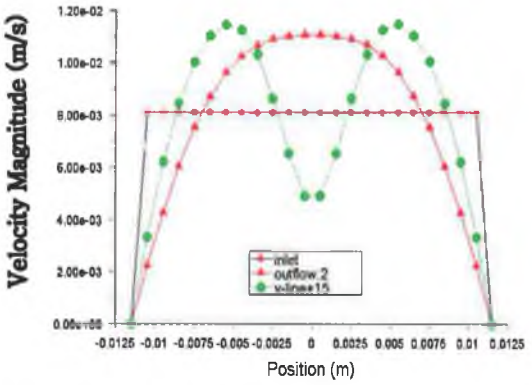


Figure 6.17.Velocity profile at plot +15

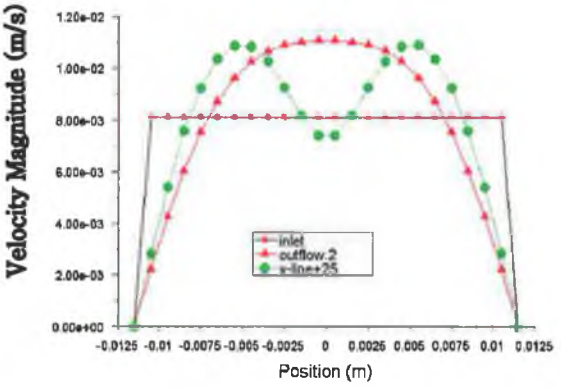


Figure 6.18 Velocity profile at plot +25

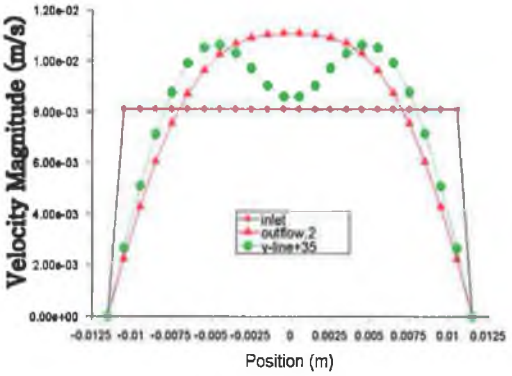


Figure 6.19. Velocity profile at +35

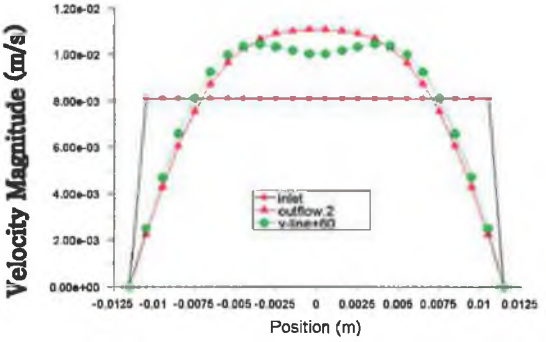


Figure 6.20. Velocity profile at +60

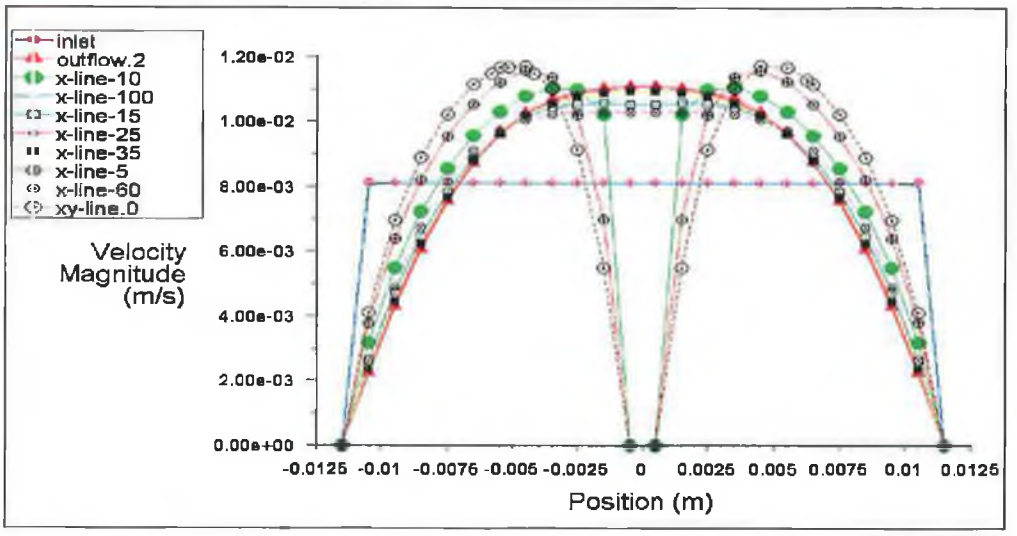


Figure 6.21.Velocity magnitude of upstream plots of 0.0081 m/s

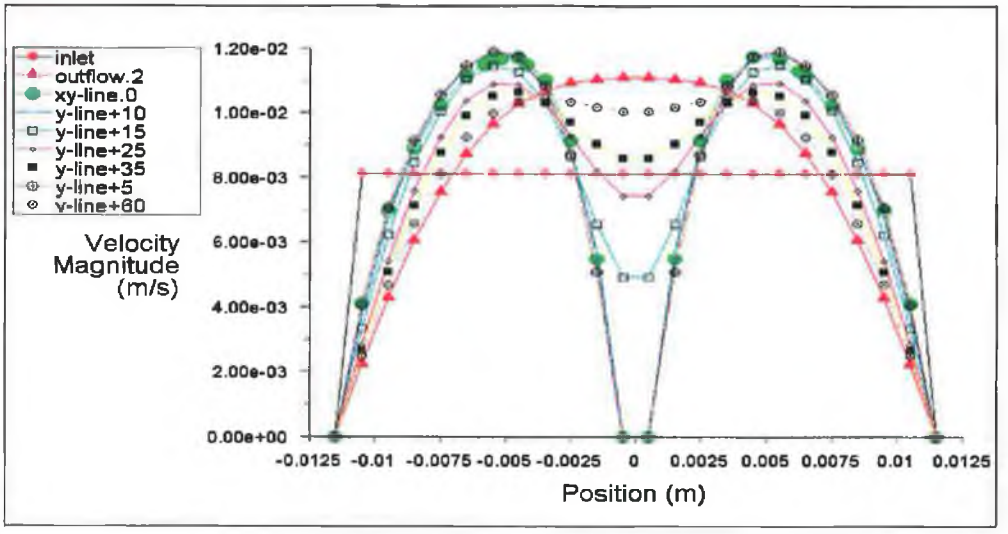


Figure 6.22. Velocity magnitude of downstream plots of 0.0081 m/s

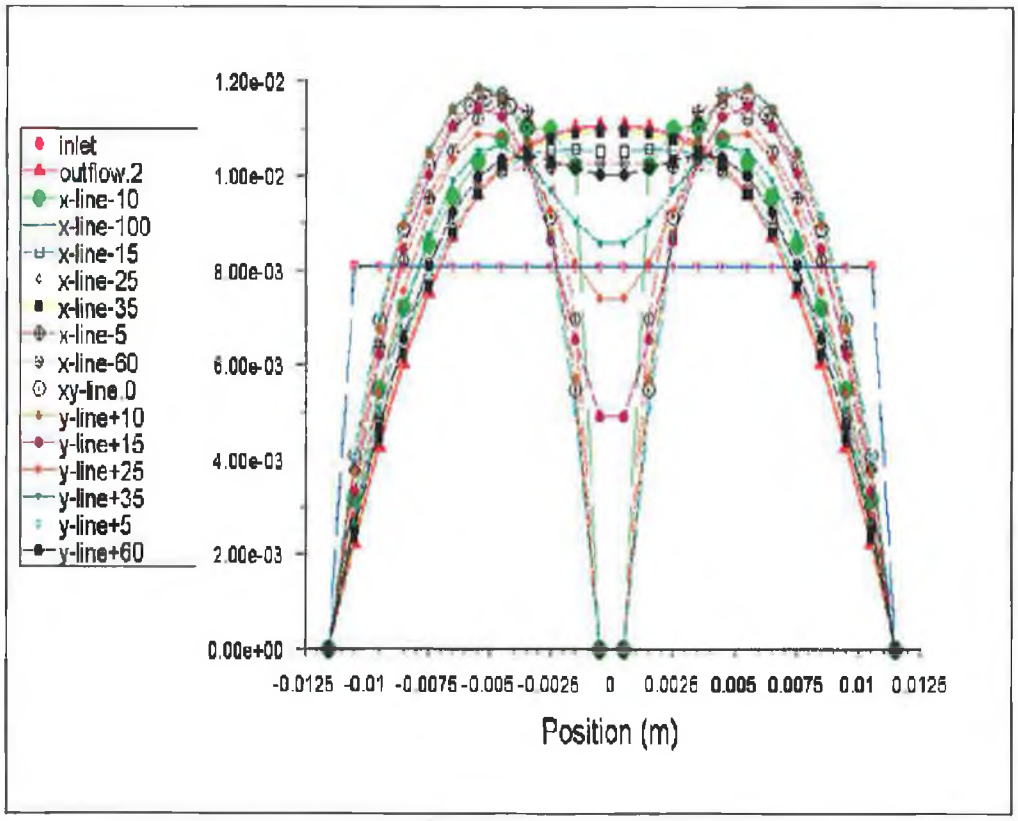


Figure 6.23. Velocity Magnitude combine upstream and downstream at0.0081 m/s

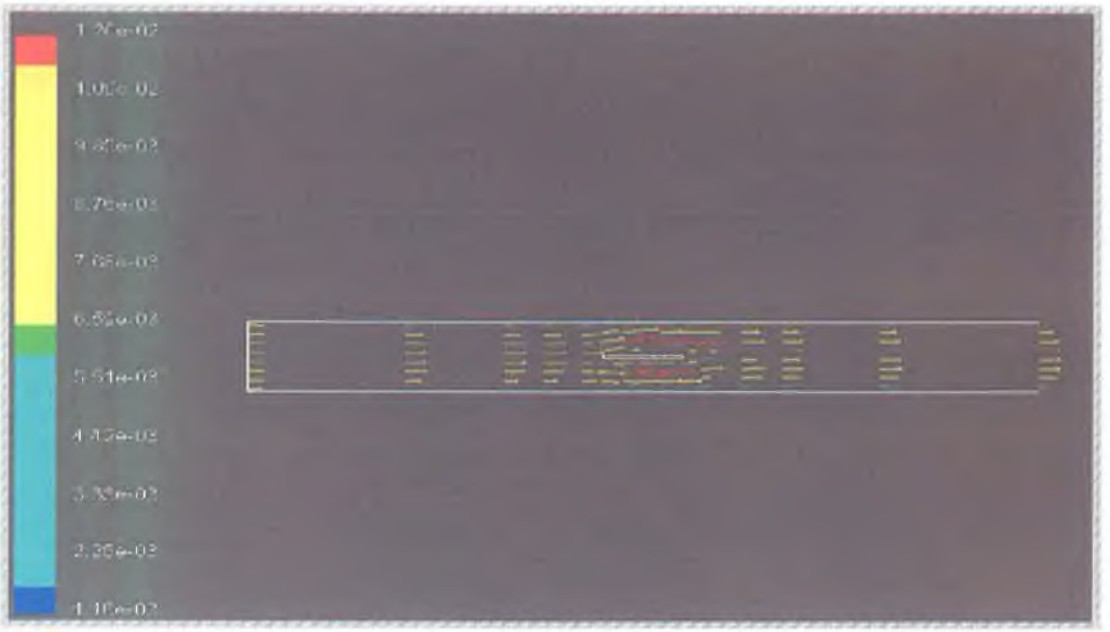


Figure 6.24. Velocity vectors at 0.0081 m/s in the pipe

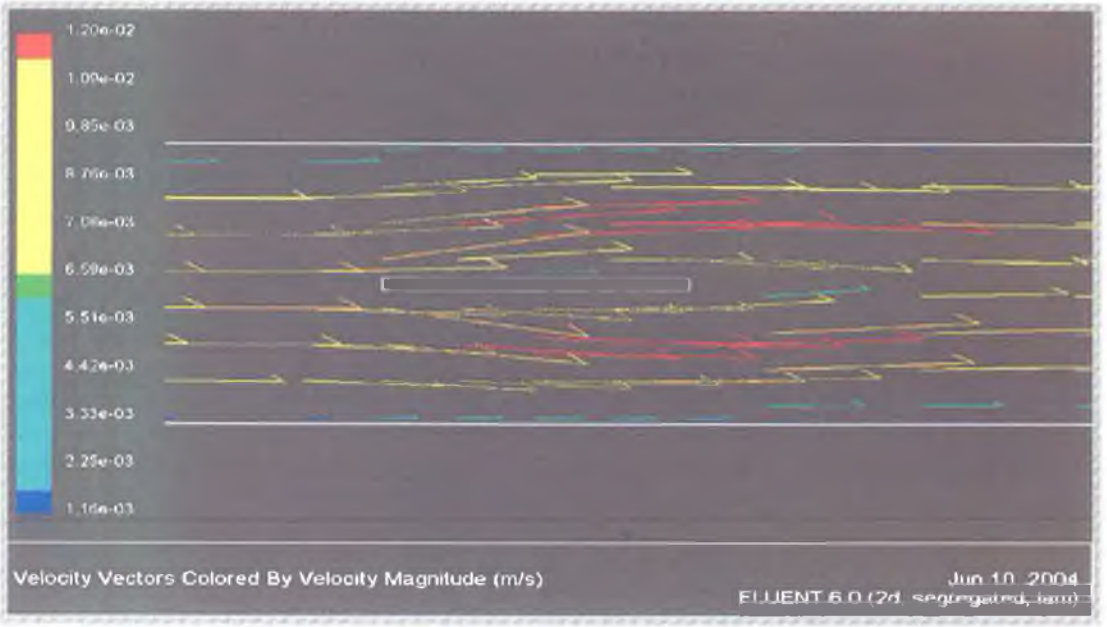


Figure 6.25. Velocity vector at 0.0081 m/s around the slide

Graphs of velocity profiles of laminar velocity (0.0081 m/s) within the tube are presented in figures (6.7 to 6.23). The plots represent the inlet of the tube at –60, -35, -25, -15, -10 (upstream) and the centre of the slide at 0 (zero). The outlet is represented at +5, +10, +15, +25, +35 and +60 (downstream). Each graph shows the flow velocity in different zones. In plot –60 the flow velocity is flat typical of a laminar flow profile. In the plot –35 and –25 the flow still detached, little change in the fluid curve up to plot -25. The plot –15 presents the pre-attached zones with semi-convex peak carves (slight anticipation of the slide upstream).

The plots –10, -5, zero, +5, and +10 presents the water flow across the slide.
In these plots the fluid velocity curve changed completely and divided into two sharp peaks; then they return back slowly to the normal profile shape at the re-attachment zones in plots +15, +25, +35 and plot +60 (the fluid exit from slide in these plots). In plot –10 at the start of the slide of first profile will move towards wall of slide, at the middle of slide (plot zero), two laminar profiles exist. Flow attempts to recover to outflow profile in plot +60.

Figures 6.21, 6.22 and 6.23 present a comparison between the water velocities slide in the upstream and downstream of the slide. It's clear from the figure (6.22) throughout the concave profile that the fluid has more attached to the downstream plots than in the upstream (where the cells counts are less; Tables 5.1, 5.2, 5.3 and 5.4)

Vectors (figures 6.24 and 6.25) present the velocity profile plots into the tube and the movement of the fluent around the slide. Generally there is no direct attachment of the fluid to the slide, and the fluent movement around the slide appears in oval shape (6.25).

The fluid movement and attachment or detachment detects the reason of cells sedimentation on the five slide zones (figure 4.10) in this laminar flow velocity. From tables (5.1, 5.2, and 5.3) the cells counts increased in the inlet zones more than in the

outlet, but were higher on the sides of the slide; the centre of the slide had the lowest cell accumulation. This may be attributed to the flow pattern of fluid across the slide with good fluid compact on the upstream face, separation across the mid-section of the slide and re-attachment on the downstream face.

6.8 Turbulent Velocities of 0.0772 m/s and Reynolds number of 1707.2

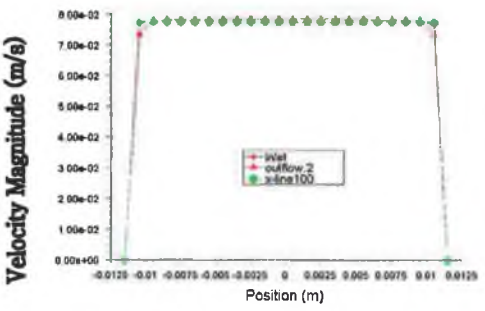


Figure 6.26. Velocity profile at -100

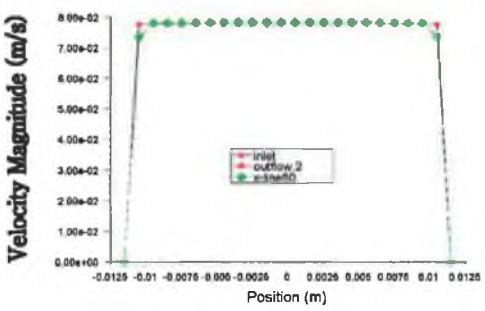


Figure 6.27. Velocity profile at-60

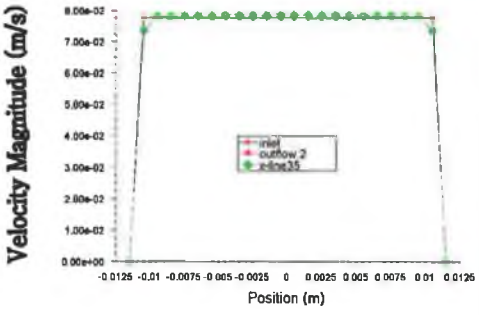


Figure 6.28. Velocity profile at -35

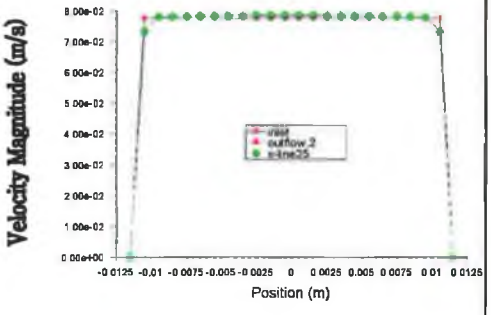


Figure 6.29. Velocity profile at -25

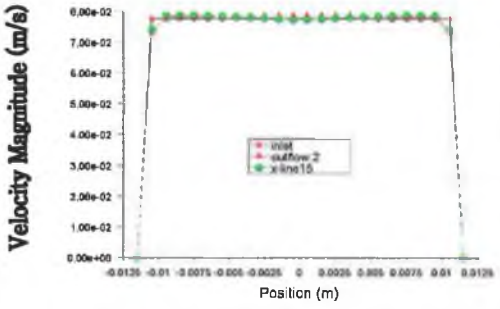


Figure 6.30. Velocity profile at -15

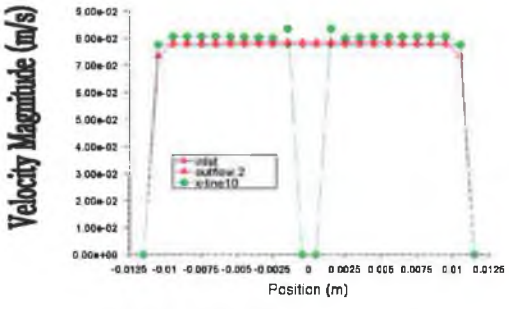


Figure 6.31. Velocity profile at -10

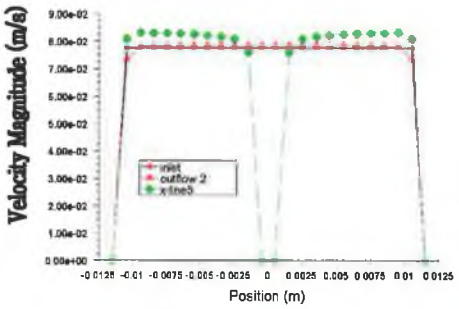


Figure 6.32. Velocity profile at –5

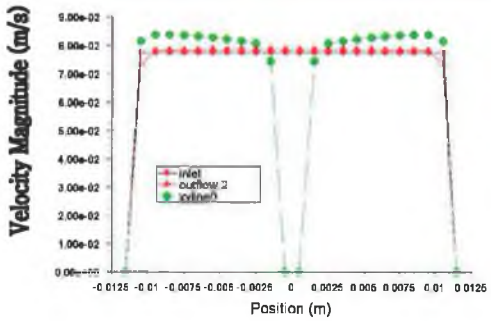


Figure 6.33. Velocity profile at zero

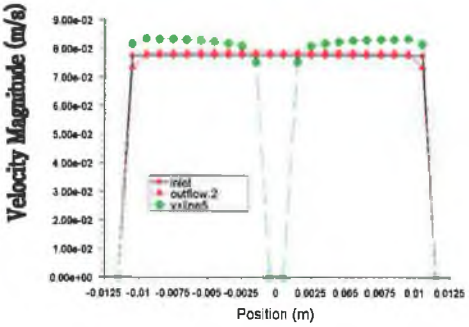


Figure 6.34. Velocity profile at +5

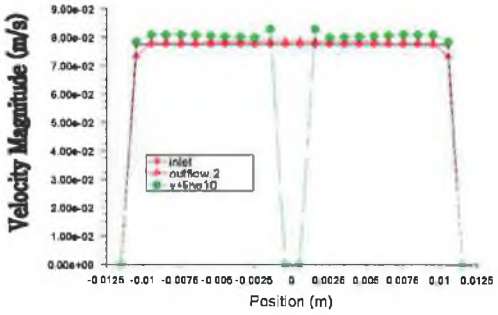


Figure 6.35. Velocity profile at +10

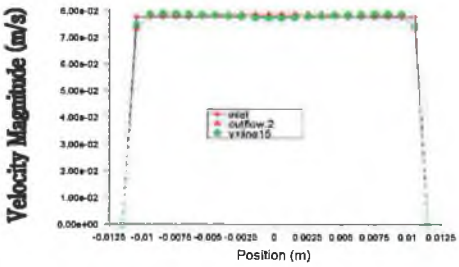


Figure 6.36. Velocity profile at +15

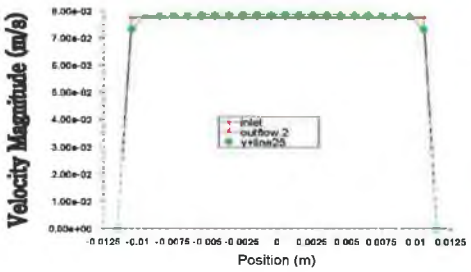


Figure 6.37. Velocity profile at +25

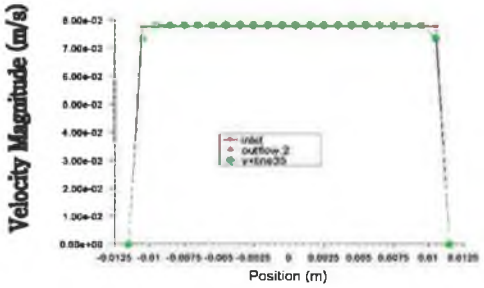


Figure 6.38. Velocity profile at +35

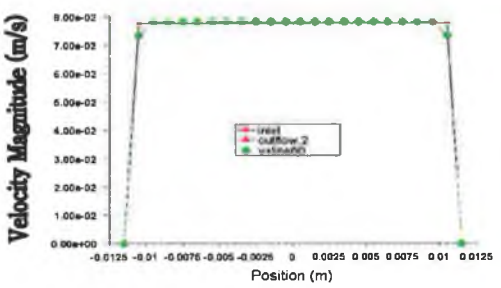


Figure 6.39. Velocity profile at +60

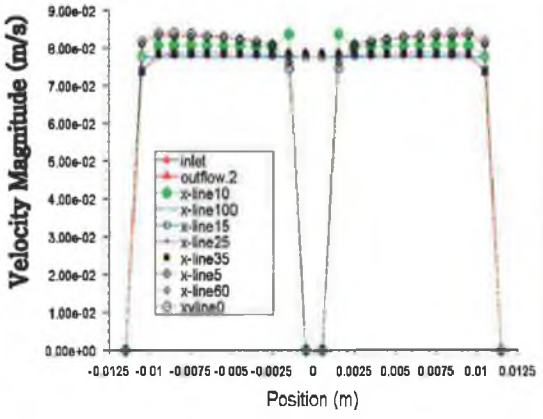


Figure 6.40.Velocity magnitude of upstream plots of 0.0776 m/s

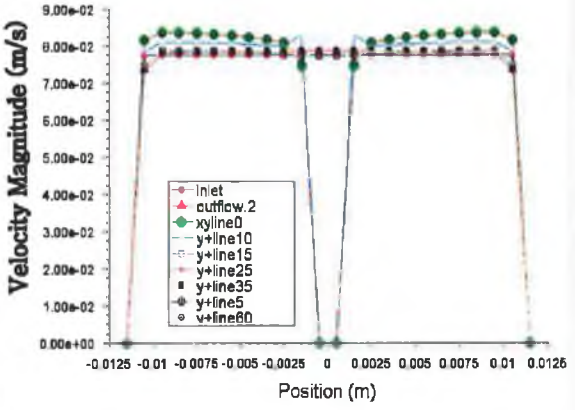


Figure 6.41.Velocity magnitude of downstream plots of 0.0776 m/s

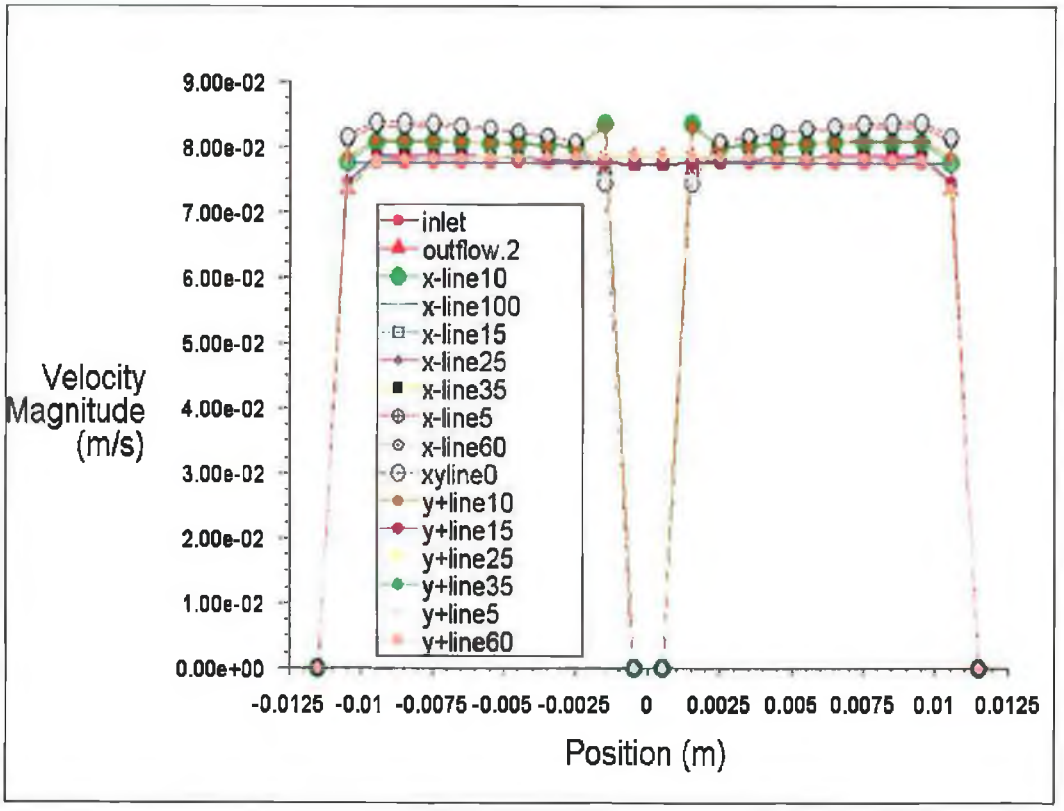


Figure 6.42. Velocity Magnitude combined upstream and downstream at 0.0776m/s

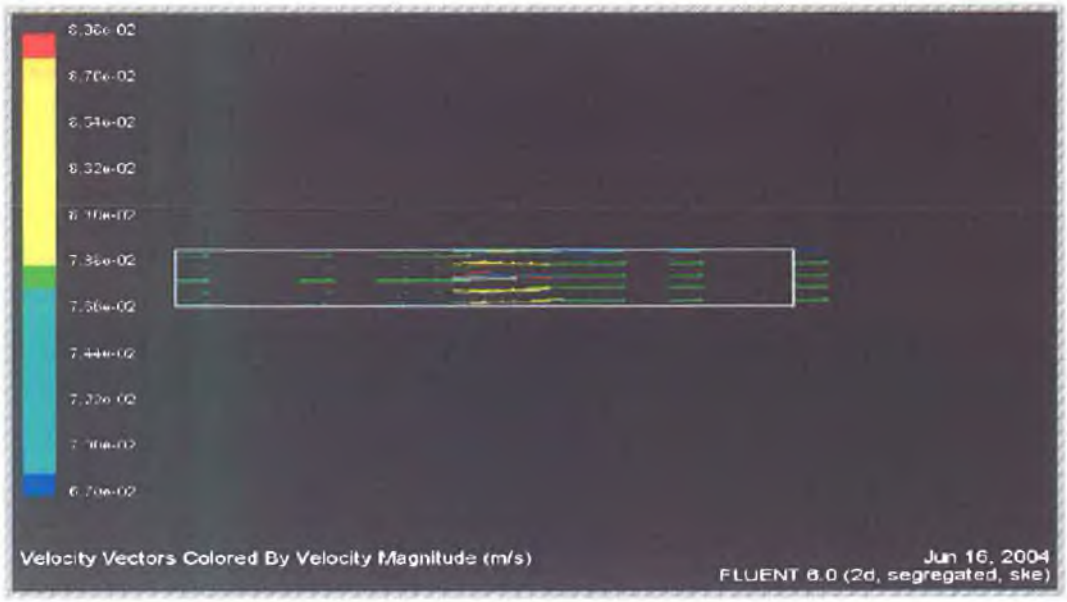


Figure 6.43. Velocity vector of 0.0776 m/s in the tube

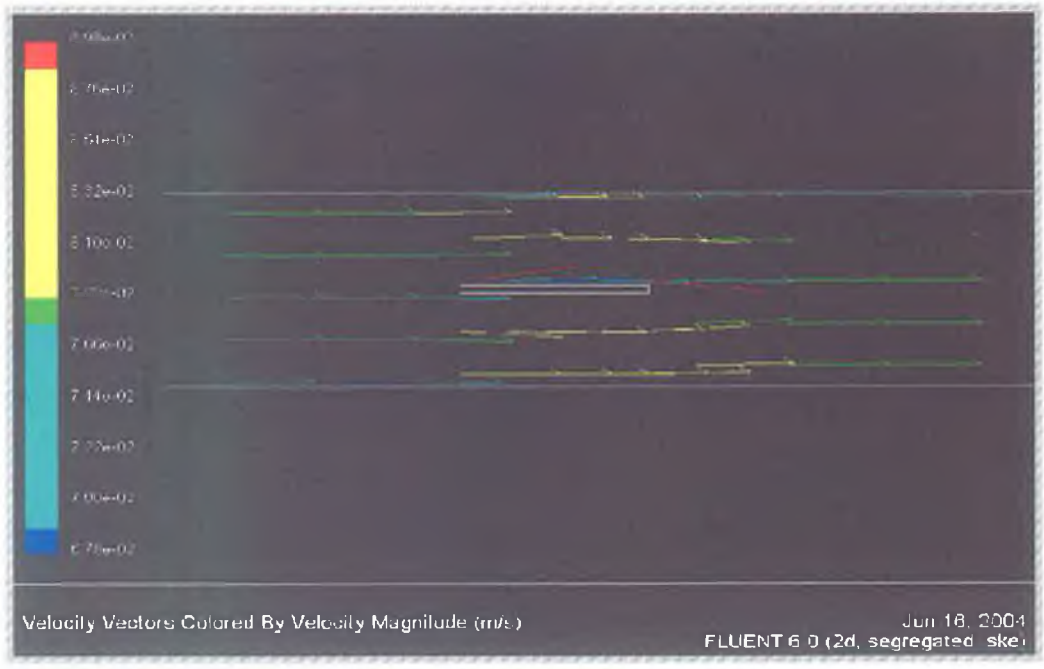


Figure 6.44. Velocity vector of 0.0776 m/s around the slide

Figure (6.26 to 6.39) presents the velocity profiles of turbulent flow at 0.0776 m/s. No change in the velocity plots in the upstream areas (-100. –60, -35, -25 and –15). Little change upstream up to plot –15, the fluid plots start to change in plot –10, the fluid velocity increase along the wall of the slide, which present the fluid attachment to the slide, the divided into tow flat peek curves with sharp edges presents the fluid attachment to the inlet side (zone 1, figure 4.10) of slide. The same curve shape was present on the outlet area (plot +10), where the fluid attached the downstream side of the slide (zone 3). From (tables 5.1,5.2 and, 5.3) the highest cell counts given in the inlet (zone 1) and outlet (zone 3) areas especially on the horizontal slides. Slight skewed profile towards the wall of the tube with decrease in velocity along the wall of the slide in plot –5. Rapid recovery of original inlet profile with little distribution downstream in plot +15. The plots -5, 0 and +5 present the fluent velocity over the centre (zone 5) of the slide and zones 2 and 4. The cell accumulation on the slid sides is less (zones 2 and 4) and rises in the centre of the slide (zone 5). The fluent velocity return immediately at outlet points of +15, +25, +35 and +60.

Figures (6.40, 6.41, and 6.42) show comparison between the upstream and downstream velocities and their change through the tube.

Figures (6.43 and 6.44) present the velocity vectors, its clear there is direct contact /attachment of the fluid to the slide.

From table (5.4) the cells count in this turbulent flow velocity is high (1051cell count), but less than the laminar flow cell count.

6.9 Turbulent Velocity Of 0.1332m/s and Reynolds number of 2930.4

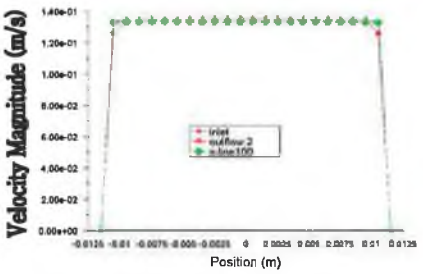


Figure 6.45. Velocity profile at plot -100

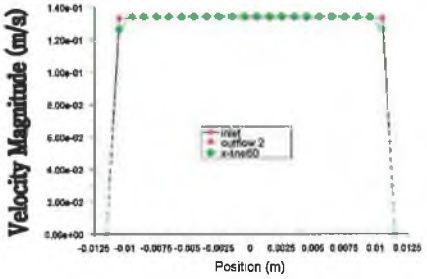


Figure 6.46. Velocity profile at plot -60

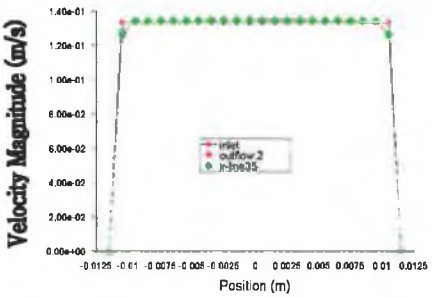


Figure 6.47. Velocity profile at plot -35

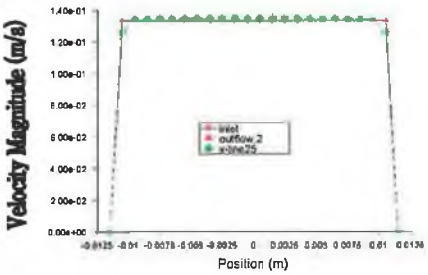


Figure 6.48. Velocity profile at plot -25

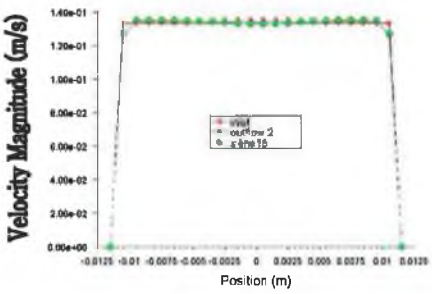


Figure 6.49. Velocity profile at plot -15

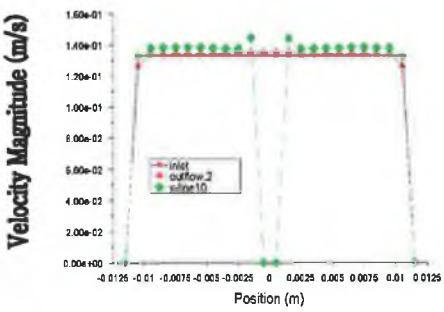


Figure 6.50. Velocity profile at plot -10

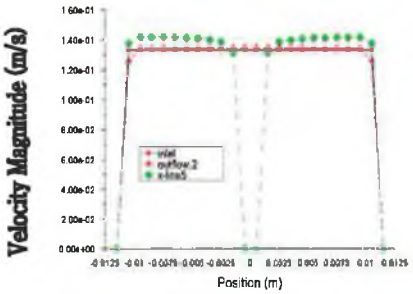


Figure 6.51. Velocity profile at plot -5

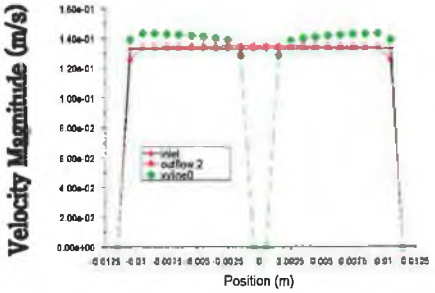


Figure 6.52. Velocity profile at plot zero

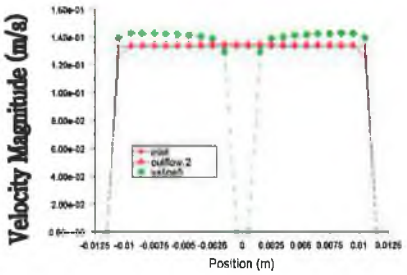


Figure 6.53. Velocity profile at plot +5

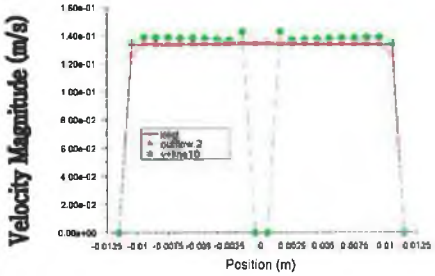


Figure 6.54Velocity profile at plot +10

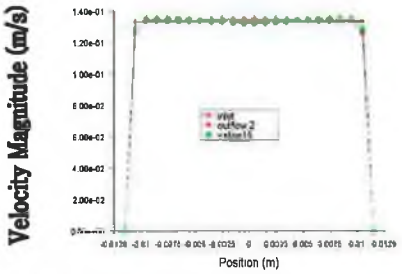


Figure 6.55Velocity profile at plot +15

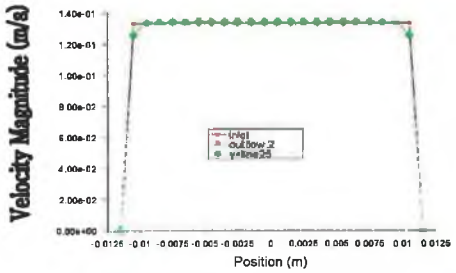


Figure 6.56. Velocity profile at plot +25

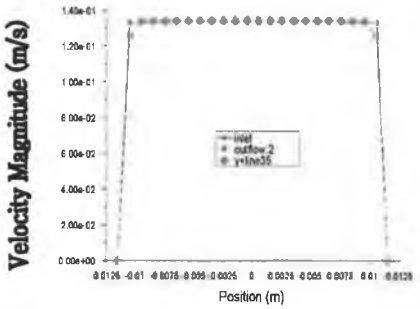


Figure 6.57. Velocity profile at plot +35

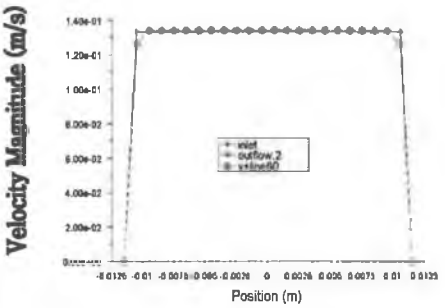


Figure 6.58Velocity profile at plot +60

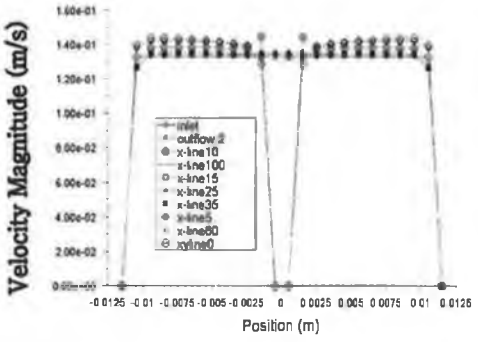


Figure 6.59.Velocity magnitude of upstream plots of 0.1332m/s

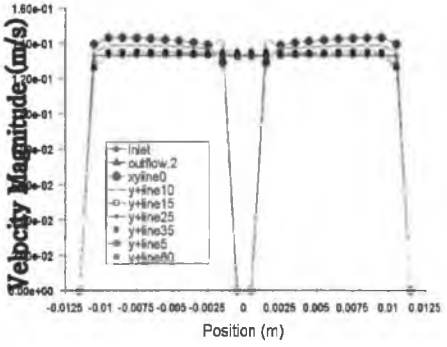


Figure 6.60.Velocity magnitude of downstream plots of 0.1332 m/s

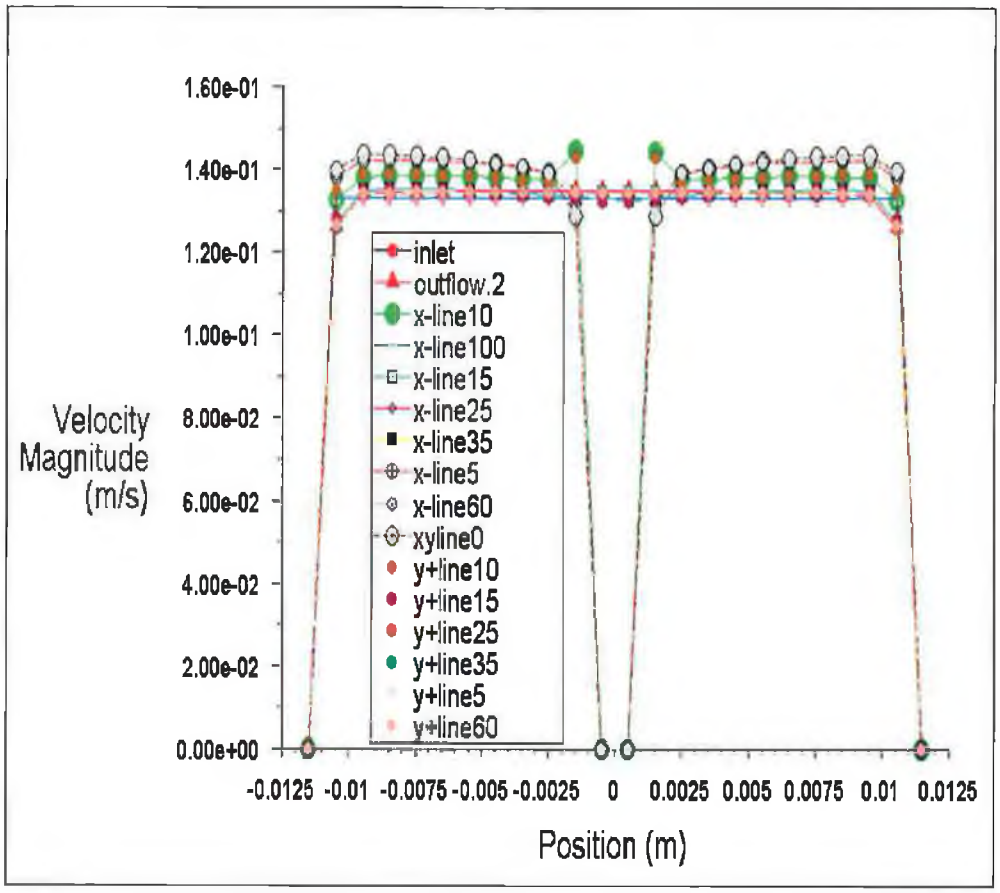


Figure 6.61. Velocity magnitude combined downstream and upstream at 0.1332m/s

Figure 6.62. Velocity vector of 0.1332 m/s in the tube

Figure 6.63. Velocity vector of 0.1332 m/s around the tube

These turbulent velocity flow profiles of 0.1332 m/s (figures 6.45 to 6.58) show no change of the previous result of the turbulent velocity flow result of velocity 0.0776 m/s. No change in the fluent curves in the inlet areas (-100. –60, -35, -25 and –15), the fluent curve start change in the plot –10, which present the fluent attachment to the slide, the divided into two flat peek curves with sharp edges again presents the fluid separation around the inlet side (zone 1, figure 4.10) of the slide. The same curve shape given in the downstream area (plot +10), where the fluent attached the outlet side of the slide (zone 3). From (tables 5.1, 5.2 and, 5.3) the cell count increased with the turbulent velocity of 0.0776 in the inlet (zone 1) and outlet (zone 3) areas especially in the horizontal slides. Plot +15 show different profile, not an immediate recovery as profile 0.0776 m/s. The plots -5, 0 and +5 present the fluent velocity over the centre (zone 5) of the slide and its sided (zones 2 and 4), The cell accumulation on slid sides is higher (zones 2 and 4) with this flow velocity but lesser in the centre of the slide (zone 5), (tables 5.1, 5.2, and 5.3). The fluent velocity return steady in outlet plots of +15, +25, +35 and +60.

Figures (6.59, 6.60, and 6.61) show comparison between the inlet and outlet velocities and their gradation through the tube.

Its clear there is direct contact /attachment of the fluid to the slide, as seen in figures (6.62 and 6.63) present the velocity vectors.

The fouling or the cell accumulation on the slide in this velocity is higher/ (raised to 25683 cell count, table 5.4) than that of the turbulent velocity of 0.0776 m/s.

6.10 Turbulent Velocity Of 0.1598 m/s and Reynolds number of 3515.6

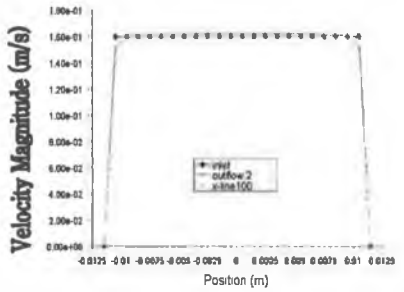


Figure 6.64.Velocity profile at plot -100

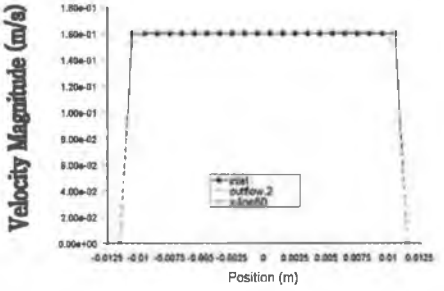


Figure 6.65.Velocity profile at plot -60

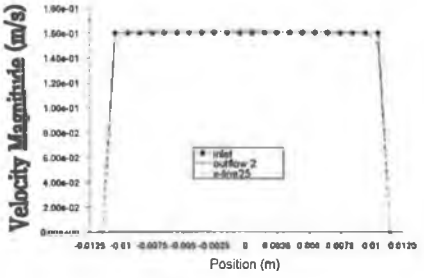


Figure 6.66.Velocity profile at plot -25

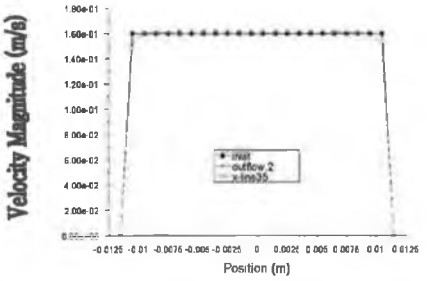


Figure 6.67.Velocity profile at plot -35

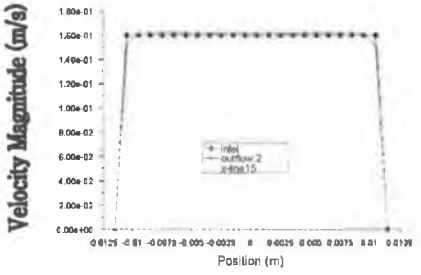


Figure 6.68.Velocity profile at plot -15

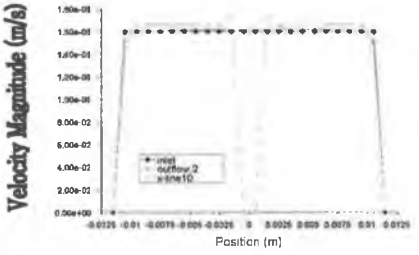


Figure 6.69.Velocity profile at plot -10

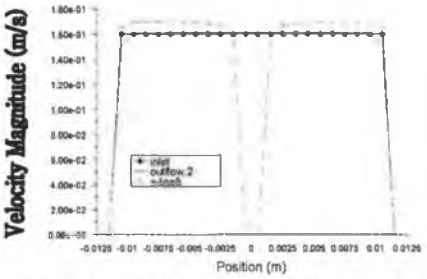


Figure 6.70.Velocity profile at plot -5

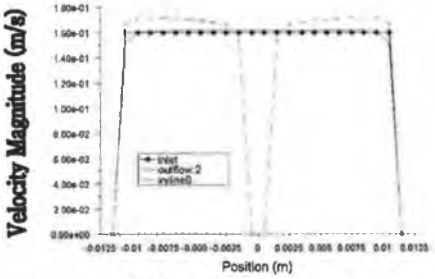


Figure 6.71.Velocity profile at plot zero

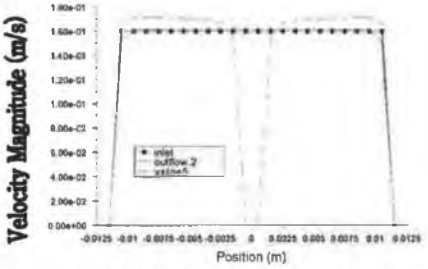


Figure 6.72.Velocity profile at plot +5

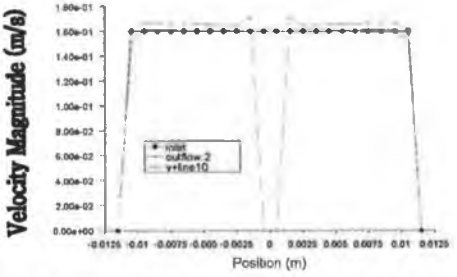


Figure 6.73.Velocity profile at plot +10

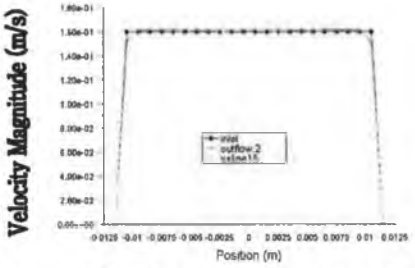


Figure 6.74.Velocity profile at plot +15

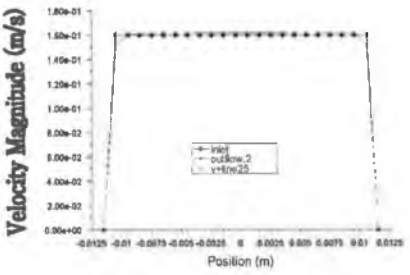


Figure 6.75.Velocity profile at plot +25

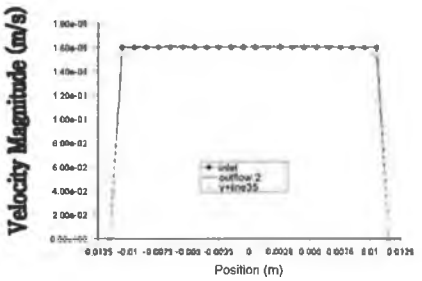


Figure 6.76.Velocity profile at plot +35

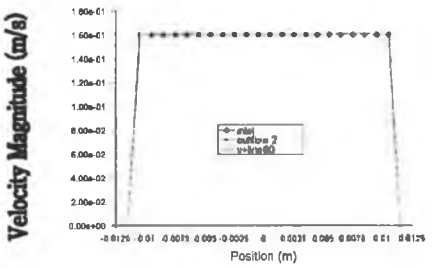


Figure 6.77. Velocity profile at plot +60

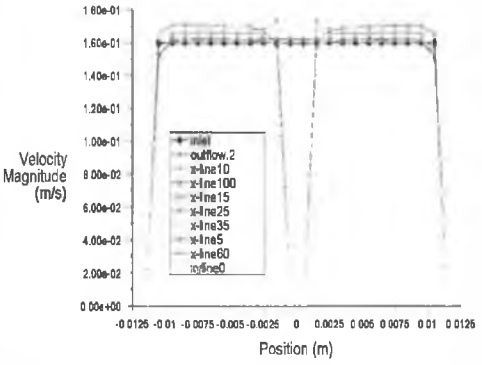


Figure 6.78.Velocity magnitude of upstream Plots Of 0.1598 m/s

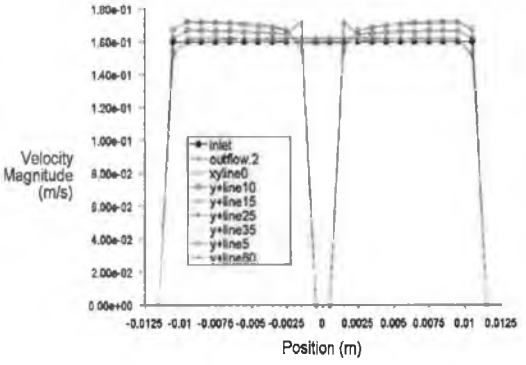


Figure 6.79.Velocity magnitude of outlet Plots Of 0.1598 m/s

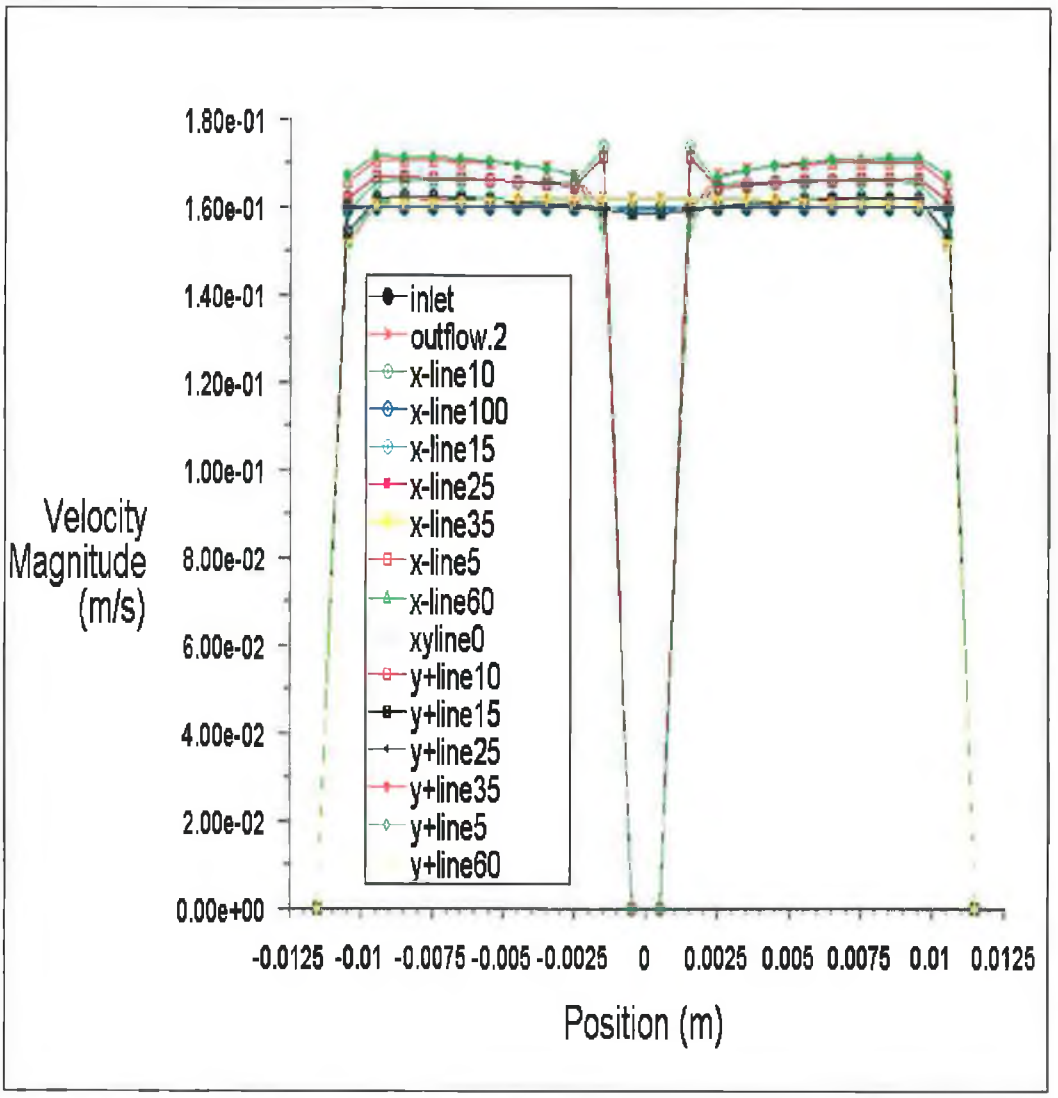


Figure 6.80. Velocity Magnitude combined upstream and downstream at o 0.1598 m/s

Figure 6.81.Velocity vector of 0.1598 m/s in the tube

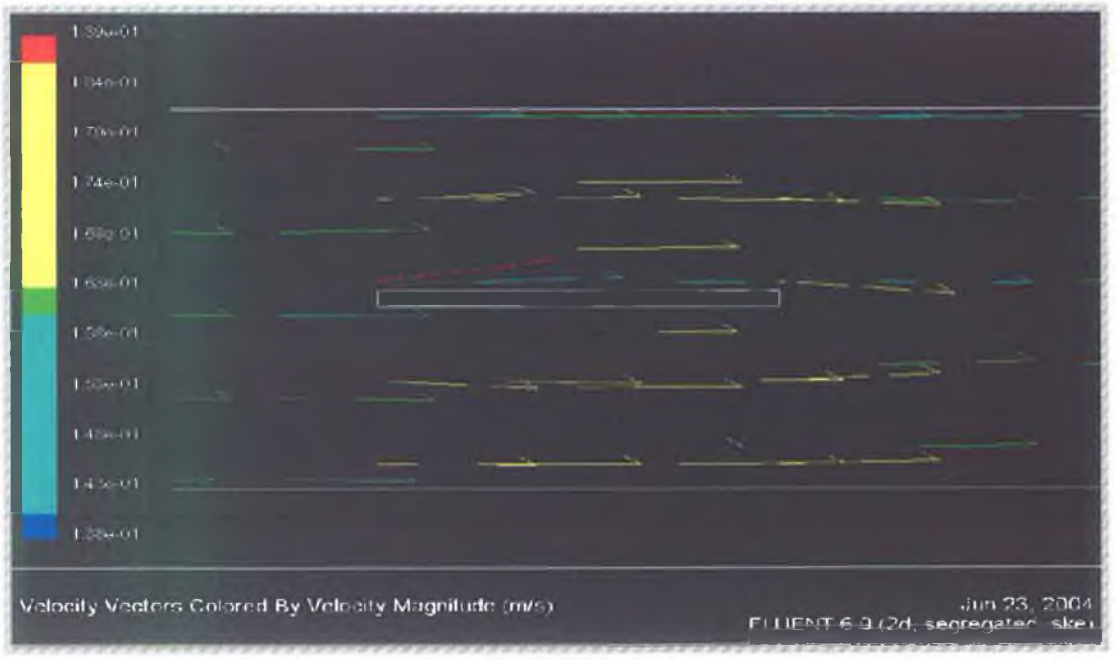


Figure 6.82. Velocity vector of 0.1598 m/s around the tube

Generally in these turbulent velocities flow of 0.1598 m/s profiles results (figures 6.64 to 6.77) there is very little change in the velocity profiles when compared to previous results of the turbulent velocities flow of 0.1332 m/s and 0.0776 m/s. Also there is little change in the fluid curves in the inlet areas (-100. –60, -35, -25 and –15), the fluid plots start change in the plot –10, which present the fluid across the slide (zone 1, figure 4.10) of the slide (increased acceleration across and around slid). The same curve shape given in the outlet area (plot +10), where the fluid attached the outlet side of the slide (zone 3). From (tables 5.1, 5.2 and, 5.3) the cell count increased more than the turbulent velocities of 0.0776 and 0.1332 m/s in the inlet (zone 1) and outlet (zone 3) areas especially in the horizontal slides. Plot –5 gives more uniform profile than in previous results, plot +10 is similar trend to all other turbulent profiles at exit from the slid and a rapid recovery again to initial inlet flow profile in plot +15. The plots -5, 0 and +5 present the fluent velocity over the centre (zone 5) of the slide and its sided (zones 2 and 4), The cell accumulation on slide sides (zones 2 and 4) is less than the inlet and outlet in this flow velocity and lesser in the centre of the slide (zone 5) than the other slide areas, (tables 5.1, 5.2, and 5.3). The fluent velocity return back steady in outlet plots of +15, +25, +35 and +60.

Figures (6.77, 6.78, and 6.79) show a comparison between the inlet and outlet velocities and their gradation through the tube.

As seen in figures (6.81 and 6.82) present the velocity vectors, its clear there is direct contact /attachment of the fluent around all the slide,

The cell accumulation on the slide in this flow velocity is more higher than the previous velocities but lesser than the turbulent velocity of 0.1332 m/s (22630 cell count, table 5.4).

6.11 General Comparison
of Laminar and Turbulent Flow

Laminar velocity profiles

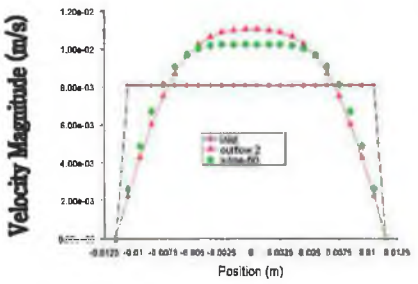


Figure 6.83.Velocity profile at -60

Turbulent velocity profiles

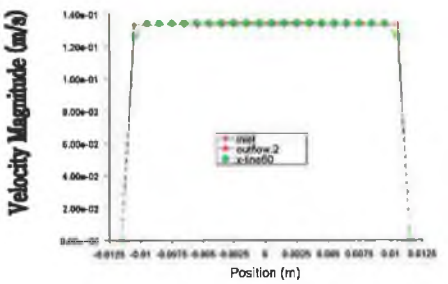


Figure 6.84.Velocity profile at +60

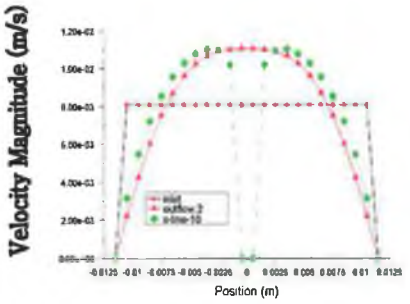


Figure 6.85.Velocity profile at -10

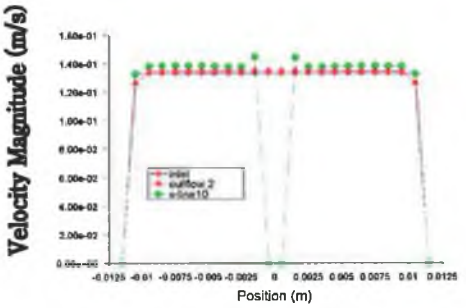


Figure 6.86.Velocity profile at +10

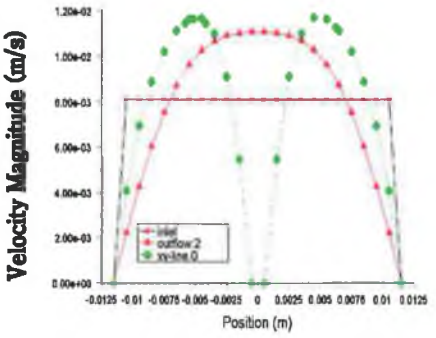


Figure 6.87.Velocity profile at zero

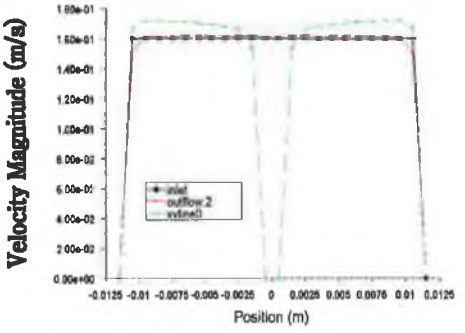


Figure 6.88.Velocity profile at zero

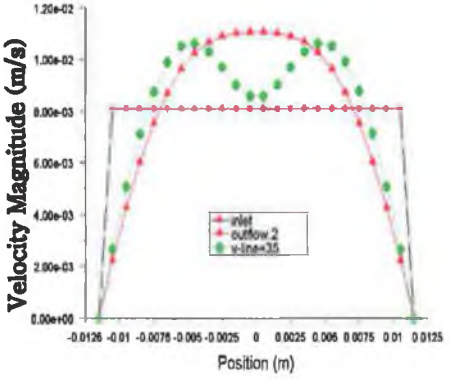


Figure 6.89.Velocity profile at -35

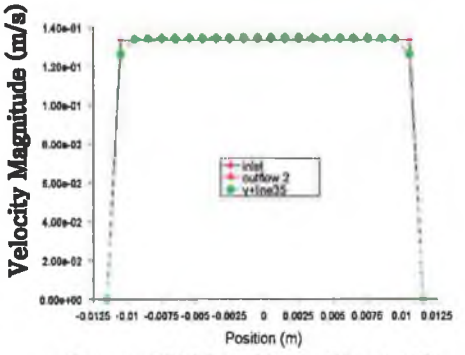


Figure 6.90.Velocity profile at +35

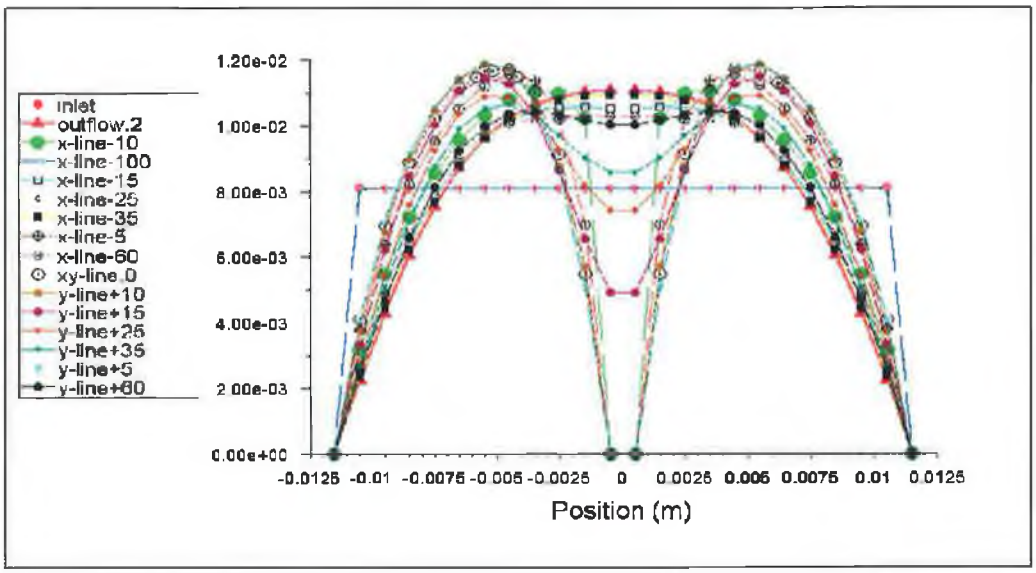
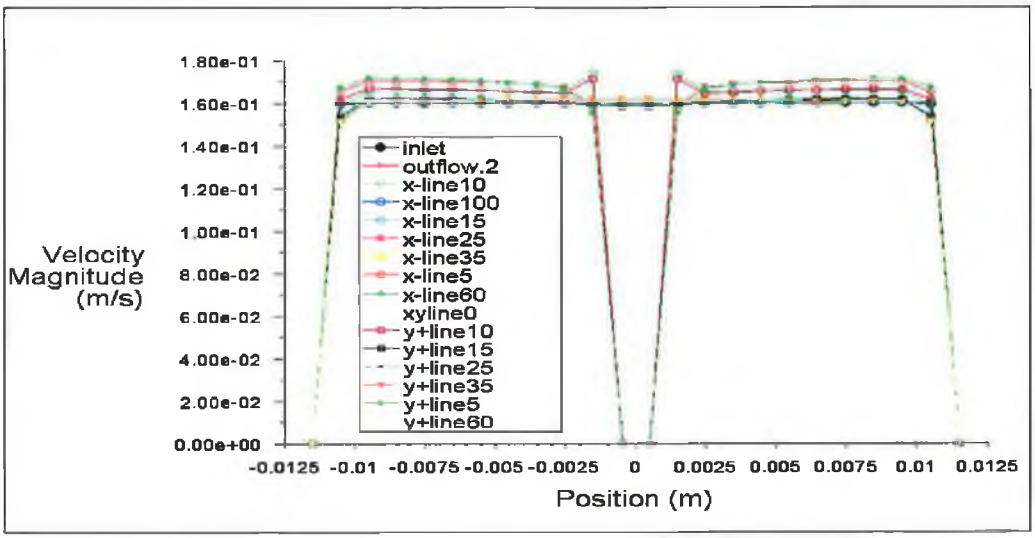


Figure 6.91.Velocities profile at upstream area



6.92. Velocities profile at downstream area

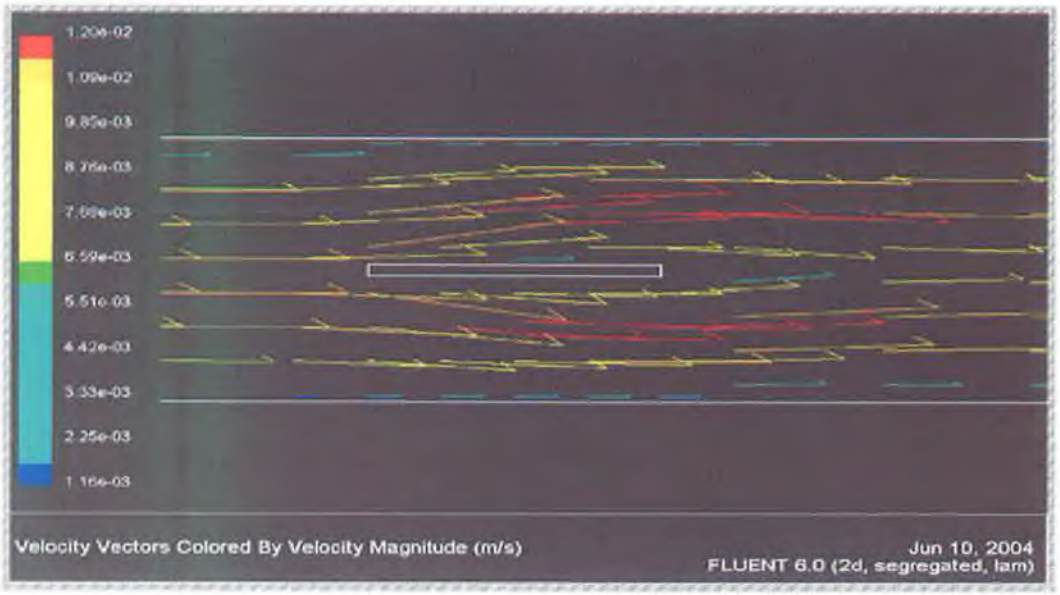


Figure 6.93. Laminar velocity (0.0081m/s)

Figure 6.94. Turbulent velocity of 0.1598 m/s

There is a difference in the velocities curves shape between the laminar velocity and the turbulent velocity. The laminar velocity curves have convex curves, where the turbulent curve has flat curves. Therefore turbulent flow gives better contact with the wall of the slide.

Note that in plots ± 10 (figures 6.85 and 6.86) where the fluid separated at the inlet side, the flow is more attached and close to the slide in the turbulent velocity (figure 6.94) represented by the sharp edges curve, than the laminar velocity. Also the centre area plots of the laminar velocity (figure 9.79), the fluid is unattached to the sides of the slide. The cell counts (tables 7.1, 7.2 and 7.3) here is less than the turbulent flow. The outlet velocity has different profile shape as seen in figures (6.91 and 6.92).

From the velocity vectors, in the laminar velocity the fluent is unattached to the slide its flow around the slide in an oval shape (figure 6.95), while the fluid in the turbulent velocities is attached to the slide around all its sides (figure 6.94) giving better opportunity for cell growth.

Chapter 7

THE CONCLUSION THE FUTURE WORK

7.1 Discussion

The Fluent software indicated the ability to be used in monitoring the biofouling of high purity water. The present data and results indicated that the fouling biofilms is higher in turbulent flow system than in the laminar flow system (table 5.4). At high flow rates, a denser, more tenacious biofilm is formed; the bacteria that accumulate tend to be of a filamentous variety especially suited for attachment by the filaments. At the higher flow rates the filamentous bacteria became a permanent part of the biofilm only after the surface had acquired great amount of extracellular material (e.g. polysaccharide), at the low flow rate, less shear stress is present and filamentous bacteria attachment slow.

The biofilms diffusion and accumulation is strongly dependent on the hydrodynamic conditions imposed by the fluid flowing over the microbial layer, at least under turbulent conditions. It would suggest that the high shear association with high velocity is sufficient to prevent any substantial accumulation of bacteria to occur; high fluid velocities corresponding to high shear stress, result in thinner, denser and more cohesive biofilms. [12].
At higher flow rates, a denser, more tenacious biofilm formed; these surfaces often appear to be free from foulants, since they are not slimy to the touch. [8].

Reynolds numbers of 1707.2, 2930.4 and 3515.6 for a given velocity corresponding to a turbulent velocity (table 5.5), it was shown that higher cell counts exist at high velocity. The level of turbulence will affect the development of the biofilm in respect to the mass transfer to and from the developing film in line with previously work of characklis (1980).

Dirik de beer and Paul Stoodley (1995) reported that mass transfer increases with increasing fluid velocity; the transport was not significant at low flow velocities. Mass transfer is roughly proportional to Reynolds number. A high degree of turbulence will facilitate the mass transfer of nutrients towards the biofilm and waste products away from the biofilm, even though at the high velocity the diffusion of nutrients and oxygen towards the biofilm would be facilitated. (M. Pujo and T.R. Bott, 1991). [20].

7.2 Conclusions

1. A rig was built to investigate biofouling in pipes. This rig incorporated glass slides and samples were taken in vertical and horizontal positions.
2. In all areas biofilm formed and different zones of accumulation we found for laminar and turbulent flow conditions.
3. Primary bacteria found were Gram negative. These were identified by a cell stain method. Maximum cells occurred at high velocity. Increasing biofilm formed with increasing flow rate.
4. Maximum cell growth was noted on the front and sides of the slides in all cases and highlight these zones as region of particular importence in the fight against biofilm.
5. A model was developed to analyse flow pattern around the slides during laminar and turbulent flow using CFD techniques.
6. The model highlighted flow separation on the upstream face of the slides and reattachment downstream of the slides.
7. These separation patterns resulted in good fluid contact with the front face of the slide and resulted in high biofilm formation.
8. Considerably different flow patterned was observed between laminar and turbulent flow, but little change was noted between high and low turbulent flow profiles.

9. Comparison between the model and experimental results show that velocity vectors at high velocity and the exchange of fluid between the water and slide surface. They can assist the development of bacteria on the surface. At low velocity the model highlights lack of contact with the slide and hence low cell counts.
10. The model shows that high velocity facilitates nutrient and oxygen circulation toward the slide which will assist cell growth as found in the experimental results.

7.3 Future work

The following is a list of recommendations for future work in this area:

1. This rig had some limitations due to the fact that it was modified from a lab based system to a research rig. A better Rig could be designed with increased entry and exit lengths and better instrumentation.
2. Increased chemical and laboratory testing could be used to further investigate the nature of the biofilm population.
3. The use of SEM techniques at different time scales could be used to highlight the attachment and formation of biofilm.
4. Flow visualisation studies could be used to highlight regions of stagnation and validate CFD studies.
5. Design of an industrial scale test-cell for use in industrial water purification system would also be of interest.

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