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Master Thesis

"Biosecurity of Gases in Aquaculture and Food Industry"

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SUMMARY (in English)

In this research we intended to investigate the role of applying Industrial gases, particularly Liquid Oxygen (LOX) and Liquid Nitrogen (LIN) in fish farming and food industries in Iran.

A brief specification, production, application and transportation of these two gases have been explained. As follows we practically applied LOX in fish farming and LIN in food industry.

Using LOX in fish farming we should report that it has been applied for the first time by our group in Iran and we faced lot of resistance again this project. Most of the fish farm owners thought it as a big risk for their farm. This was based to their unwariness of how to use the oxygen supply system to the rearing water in their farms and also the possible effect of such enriched in oxygen water in the fish farms. Being not familiar with this subject, they were afraid of losing their fishes as well as the whole benefit of raring fishes on that specific farming period. To a certain degree, they had reasons to be afraid since some fish diseases occurred or trigged due to the high level of dissolved gasses in water, as for example the bobble disease in trout. At the end we convinced a fish farmer in Iran to initiate the project. Thus, we installed a bio-secure system of oxygen enrichment in their farms and the result was incredibly good in growth rate and survival of their fish.

Despit the difficulties which we encountered to apply liquid oxygen in fish farm, the use of liquid nitrogen in food industry is already a common procedure in Iran. This enabled us to apply this gas in food and observe the corresponding results. More specific, in our study we investicated the effect of applying Liquid Nitrogen for packing eidable oil in bottles. The result here was also very useful

and beneficial for the eidable oil companies as oil has a longer self-life and the final product is cost effective too.

Our study encourages the further use of gases in Iran and proved that not only the food industry but also the aquaculture industry has many benefits in using gases, which in turn are also absolutely bio-secure for the humans and the rearing animals – fishes.

SUMMARY (in Greek)

Στην παρούσα εργασία εξετάσαμε την δυνατότητα χρήσης βιομηχανικών αερίων και ειδικότερα του Υγρού Οξυγόνου (LOX) και του Υγρού Αζώτου (LIN) στην ιχθυοκαλλιέργεια και την βιομηχανία τροφίμων στο Ιράν.

Αρχικά πραγματοποιήσαμε μια σύντομη αναφορά στις ιδιότητες, τρόπο παραγωγής, εφαρμογή και μεταφορά των δύο αυτών αερίων. Στην συνέχεια αναφερθήκαμε στην πρακτική εφαρμογή των στις μονάδες παραγωγής ψαριών και στην συντήρηση των τροφίμων.

Αναφορικά με την χρήση του Υγρού Οξυγόνου στην ιχθυοκαλλιέργεια, πρέπει να αναφερθεί ότι για πρώτη φορά εμείς πραγματοποιήσαμε την συστηματική και όχι περιστασιακή – επικουρική χρήση του στο Ιράν και για τον λόγο αυτό αντιμετωπίσαμε πολλές δυσκολίες κατά την εκπόνηση της εργασίας μας. Οι περισσότεροι ιχθυοκαλλιεργητές θεώρησαν την χρήση του ως πιθανό μεγάλο κίνδυνο για τις μονάδες τους. Αυτό οφείλονταν στην μέχρι τώρα άγνοια (από την πλευρά των ιχθυοκαλλιεργητών) σχετικά με τον τρόπο χρήσης ασφαλούς συστήματος παροχής οξυγόνου στο νερό εκτροφής των μονάδων τους και του αποτελέσματος που θα είχε το εμπλουτισμένο σε οξυγόνο νερό σε αυτές. Μη εξοικειωμένοι λοιπόν με τα ανωτέρω, φοβόταν ότι θα προκαλούσαν πιθανώς κακό στα ψάρια τους και συνεπώς θα έχαναν την παραγωγή τους κατά την συγκεκριμένη ιχθυοκαλλιεργητική περίοδο. Έως κάποιο βαθμό είχαν λόγους να είναι επιφυλακτικοί διότι ορισμένες ασθένειες προκαλούνται ή/και ενεργοποιούνται λόγω της ύπαρξης αερίων διαλυμένων σε υψηλές συγκεντρώσεις στο νερό εκτροφής, όπως για παράδειγμα συμβαίνει στην περίπτωση της νόσου των φυσαλίδων στην πέστροφα. Τελικά, πείσαμε μία μεγάλη μονάδα παραγωγής πέστροφας στο Ιράν να ακολουθήσει την εφαρμογή

του προτεινόμενου από εμάς προγράμματος εμπλουτισμού του νερού της με οξυγόνο. Ακολούθως, εγκαταστήσαμε ένα σύστημα απόλυτα βιο-ασφαλές για τον εμπλουτισμό του νερού με οξυγόνο και τα αποτελέσματα ήταν εντυπωσιακώς βελτιωμένα ως προς τον ρυθμό ανάπτυξης των ψαριών, την επιβίωση τους καθώς και την αύξηση του ιχθυοπληθυσμού τους κατά 43% στις ίδιες (υπάρχουσες) δεξαμενές εκτροφής.

Σε αντίθεση με τις δυσκολίες που αντιμετωπίσαμε στην χρήση του υγρού οξυγόνου στην ιχθυοκαλλιέργεια, η χρήση του υγρού αζώτου ήταν ήδη συνήθης στην βιομηχανία τροφίμων στο Ιράν. Το γεγονός αυτό μας έδωσε την δυνατότητα να το χρησιμοποιήσουμε στα τρόφιμα και να παρατηρήσουμε τα αντίστοιχα αποτελέσματα. Ειδικότερα, στην εργασία μας διερευνήσαμε το αποτέλεσμα της χρήσης υγρού αζώτου κατά την συσκευασία εδώδημου ελαίου σε φιάλες. Τα αποτελέσματα ήταν ιδιαίτερα χρήσημα για τις αντίστοιχες εταιρείες συσκευασίας ελαίου διότι παρέτεινε την περίοδο κατανάλωσης και μείωνε το κόστος παραγωγής του συσκευασμένου τελικού προϊόντος.

Η εργασία μας ενθάρρυνε την ευρύτερη χρήση των αερίων στο Ιράν προτείνοντας ότι η χρήση τους όχι μόνο στην βιομηχανία τροφίμων αλλά επίσης και στην ιχθυοακαλλιέργεια έχει πολλά πλεονεκτήματα και είναι απόλυτα βιο-ασφαλής τόσο για τον άνθρωπο όσο και για τα εκτροφόμενα ψάρια.

SUMMARY (In Farsi)

در این تحقیق، نقش گازهای صنعتی بویژه اکسیژن و نیتروژن مایع در صنعت شیلات (پرورش ماهی) و صنایع غذایی مورد مطالعه و بررسی قرار گرفت.

مختصری در ارتباط با ویژگیها، نحوه تولید، نوع کاربرد در صنایع مختلف و نحوه حمل و نقل این دو گاز توضیح داده شده و نهایتا بطور عملی کاربرد اکسیژن مایع در صنعت شیلات و نیتروژن مایع در صنایع غذایی مورد مطالعه قرار گرفت .

از آنجا که تزریق اکسیژن مایع برای اولین بار در مزارع پرورش ماهی در ایران توسط این گروه انجام شد، با مخالفتهای زیادی جهت اجراء و ادامه این پروژه از طرف پرورش دهندگان مواجه شدیم. اکثر پرورش دهندگان بدلیل عدم آشنایی با سیستم تزریق اکسیژن مایع در استخرهای پرورش ماهی، از مرگ ماهیها و همچنین از دست دادن سود ناشی از پرورش درطول اجراء پروژه نگران بودند. نهایتا توانستیم یک پرورش دهنده ماهی در استان فارس را متقاعد به اجراء پروژه کنیم که نتیجه حاصله بسیار عالی بود.

علی الرغم مشکلات زیادی که در صنعت شیلات جهت ارایه و اجراء پروژه داشتیم و از آنجا که استفاده از نیتروژن مایع در صنایع غذایی از سالیان قبل مرسوم بوده است، براحتی تحقیق خود در زمینه تزریق نیتروژن مایع در بسته بندی روغن مایع را آغاز کردیم. نتایج بدست آمده در این صنعت نیز بسیار کارآمد و از نظراقتصادی مقرون بصرفه تر از شیوه های پیشین بود.

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INTRODUCTION:

As welding, diving and medicine production are the situation where the gas is seen in operation, we mostly consider them as the only gas application in Industry. The fact is that gases have a very wide field of applications, and they are used somewhere in almost every production chain.

As market demand grows and as the new technology get more expand, gases find their way into new application felids. Most pure gases with different purities and a huge variety of gas mixtures are applied in our daily life [1].

Automotive, Chemistry, Clean Energy, Food, Aquaculture, Manufacture, Medical, Metal Industry, Pharmaceutical, OEM (Original Equipment Manufacturer), Petrochemical, R & D (Research & Development) are some industries which definitely depend on using of Industrial gases.

In this study we mainly focus on Liquid Oxygen (LOX) and Liquid Nitrogen (LIN) as two main Industrial gases in Food and Aquaculture industries. The properties, production and the biosecurity of applying these two gases in food and Aquaculture industry will be investigated.

CHAPTER 1: INDUSTRIAL GASES:

As applying Industrial gases is essential in our daily life, to be familiar with the specification and characteristic of them is also necessary for us. The more we know about the industrial gases, the more application will be innovating to make our life easier.

In this research, among all the different industrial gases we focus on Oxygen and Nitrogen, their characteristic and the biosecurity effect of them in fish farm and food industry.

1.1: Oxygen:

This chapter is discussed about physical and chemical properties of Oxygen and Nitrogen as well as production, storing and transportation of these two gases. The wide range of these gases application in different industries will be introduced.

1.1.1: Air Composition:

The physical environment of our planet earth is divided into three sections: Atmosphere - the gaseous envelop of the earth -, the solid earth (Lithosphere) and the water portion of planet (Hydrosphere). Atmosphere as the gaseous part of earth plays an important role in earth physical environment, because it is in a continuous interaction with the two other parts of earth physical environment: The solid earth (Lithosphere) and the water portion of planet (Hydrosphere) [2].

The life form of planet – Biosphere – is also connected with each of the three physical spheres. The interplay and interaction among the domains of earth make it a proper environment to live in.

Atmosphere is able to control the energy distribution over the earth's surface, as it's in a close contact with the oceans, the biosphere, and the lithosphere, from one side and on the other hand with the solar system and space. Also, it is a capable medium to transfer material from one sphere to another one [3]. Therefore, it's very critical to be familiar with the compositions of Atmosphere. (Table1.1.1).

Air composition of Atmosphere is based on below Table 1.1.1.

Table 1.1.1,: Composition of the atmosphere at ground level [(based on Junge, 1963; Andrews et al., 1966; IPCC report 2001).

Residence time	Concentration % or ppm	Gas
–	78.084%	**Nitrogen (N_2)**
–	20.946%	**Oxygen (O_2)**
–	0.934%	**Argon (Ar)**
10 days	(0.4 to 400) J 10^2 ppm	**Water (H_2O)**
4 yrs	370 ppm (280 ppm)*	**Carbon dioxide (CO_2)**
–	18.18 ppm	**Neon (Ne)**
~$2\cdot10^6$ yrs	5.24 ppm	**Helium (He)**
~10 yrs	1.75 ppm (0.7 ppm)**	**Methane (CH_4)**
–	1.14 ppm	**Krypton (Kr)**
–	0.4 to 1.0 ppm (??)	**Hydrogen (H_2)**
–	0.087 ppm	**Xenon (Xe)**

* Value at 01/01/2001 (pre-industrial value in parenthesis)

** Value at 01/01/2001 (pre-industrial value in parenthesis)

As it's shown in Table 1.1.1,. air is composed of approximately 21% Oxygen and 79% Nitrogen by volume. Knowledge of air composition has fundamentally changed human being's life. It also has had an enormous influence on the industrial development. The motor engine and truck, the airplane, engine powered vessel: e.g. ships, rockets, central heating systems, gas cookers and high explosives are some examples which none of them would have been possible by human, without knowledge of air composition [4].

Hereafter, we mainly focus on Oxygen and Nitrogen as the most composed component of Atmosphere.

1.1.1. a: Atmospheric Oxygen:

As the main source of free oxygen on earth is photosynthesis, without life there would be very little free oxygen at the Earth's surface. Based on Earth history, oxygen has increased from slight concentrations of ~ 0.21 atm (~ 21% of the present atmosphere), in a series of steps. Therefore, it has to be considered that the evolution of life influenced on oxygen and oxygen on life evolution. The only planet in the solar system which contains significant amount of free oxygen is our planet earth, and it may causes earth the only planet on which life (or at least advanced form of life) has been developed [5].

1.1.1.b: Oxygen Discovery :

On 1st August 1786 Joseph Priestley - the British chemist - [1733-1804], discovered a new gas by heating mercury in air. He found that mercury combined with part of the air to form a solid which he called red calx of Mercury. When Priestley heated red calx of mercury, he gained mercury and a new gas, which when he breathed the gas, it produced a light and easy sensation in his chest. The gas was oxygen!!! [6].

Undoubtedly, the discovery of oxygen is one of the greatest victories of modern science. With it scientists came to the conclusion that the air is a mixture of gases, 21% oxygen and 79% Nitrogen by volume [4].

Oxygen is combined widely in compounds of the earth's crust, such as inside of the mineral oxides and water (89%). Even 65% of human body is oxygen by mass [7].

1.1.1.c : Oxygen Properties :

Oxygen with symbol **O** and atomic number **16** contain 20.946% of atmosphere by volume. Oxygen is an odorless and colorless gas, with a density greater than air, and a very low solubility in water. The most remarkable chemical properties of oxygen are its ability to support respiration in plants and animals, also its ability to support combustion. It is a nonflammable gas, though the substances which are flammable in air, burn far more powerfully mixed with oxygen [7].

In other words, Oxygen does not burn or will not explode alone, though it will make a flame hotter which leads more vigorously burning. Oxygen increases the combustion speed and decreases the flash-point Temperature [1].

Free elemental oxygen occurs naturally as a gas in the form of diatomic molecules, O_2. Molecular weight of Oxygen is 31.999 and its boiling point at 1.013 bar is -182.98 Ċ.

Continuous inhalation of concentration more than 75% may cause respiratory difficulty and paroxysm, dizziness and nausea.

1.1.1.d Liquid Oxygen:

Liquid oxygen is oxygen in a liquid state with very low temperature. It has a pale blue color and is strongly paramagnetic and can be suspended between the poles of a powerful horseshoe magnet [8]. Liquid oxygen if often abbreviation as "LOX".

Cryogenic Liquid oxygen has a density of 1.141 g/cm^3 (1.141 kg/L) and is cryogenic with a freezing point of 50.5 K (−368.77 °F; −222.65 °C) and a boiling point of 90.19 K (−297.33 °F, −182.96 °C) at 101.325 kPa (760 mmHg). Liquid oxygen has an expansion ratio of 1:861 at 20 °C (68 °F), and because of this, it is used in some commercial and military aircraft as a source of breathing oxygen [9].

Because of its cryogenic nature, liquid oxygen can cause the materials it touches to become extremely brittle. Liquid oxygen is also a very powerful oxidizing agent; organic materials will burn rapidly and energetically in liquid oxygen. Further, if soaked in liquid oxygen, some materials such as coal briquettes, carbon black, etc., can detonate unpredictably from sources of ignition such as flames, sparks or impact from light blows. Petrochemicals often exhibit this behavior, including asphalt.

1.1.2: Application of oxygen in Industry:

The major uses of oxygen are based on its combustion-sustaining, oxidizing and life-supporting attributes. Whether used directly or to enrich combustion air, oxygen is widely used with fuel gases in furnaces, smelters, kilns, welding and metal cutting. Oxygen is used in chemical production as a raw material and in pulp manufacturing as a bleaching agent. Most importantly, oxygen is used for medical reasons to save and protect life [10].

Oxygen is used widely in medicine, mostly in modern anesthetic techniques including pre and postoperative managements. To heal the tensed human's tissue by improving oxygen in critical conditions such as: shock, hyperpyrexia, severe hemorrhage and coronary occlusion, carbon monoxide poisoning, road traffic accident, gunshot wounds, sudden cardiac and respiratory arrest and neo-natal recovery [11]. In pharmaceutical and chemical industry it is used for flame sealing of glass ampules for finished products. In some cases Oxygen either pure or in mixture with Nitrogen or / and carbon dioxide is used in food industry as a part of modified atmosphere package (MAP) in order to increase the shelf-life of packaged food and decrease the risk of food decay. Oxygen is used extensively for shipping live fish and other seafood to create a proper medium in order to decrease the mortality rate of these animals. In deep underwater diving mixture of o2 and He or mixture of oxygen and Nitrogen is used as a breathable atmosphere. Liquid Oxygen (LOX) explosive is used as a comburent in space propulsion. Among many Industries such as the steel, non-ferrous, glass and concrete factories oxygen is used as a supplement or replaces air in burners to achieve increased temperature. Injecting Oxygen into sewage treatment plants increases the speed rate of sewage decomposition. In order to keep an appropriate carbon balance in the CO_2 gas coolant in the nuclear reactor's core, in Advanced gas cooled (AGR), high purity Oxygen in conjunction with high purity methane is used.

Cutting, welding, chemical synthesis, Calibration gas, metal treating application, optical fiber production process and enrichment of air during fermentation are some of Oxygen application among its wide range of usage in different industries [1].

1.1.3:Production and transportation of oxygen:

Oxygen, nitrogen and argon are recovered from air using a cryogenic method developed by Carl von Linde more than 100 years ago. Particulates are removed from the incoming air, which is then repeatedly compressed and cooled. Water, hydrocarbons and carbon dioxide are then removed. With further processing, the air eventually becomes a liquid and the individual gases are separated by distillation. These plants are called air separation units, or Air Separation Units (ASU) [Linde gas pdf].

Oxygen based on its application either as chemical or scientific component categorized in two main groups. Oxygen with the purity between 99.95% and 99.9999%,and Oxygen with the purity above 99.9999%.

Oxygen with purity between 99.95% and 99.9999% is considered as chemical oxygen which is used in many different Industries and Oxygen with purity above 99.9999% which is scientific oxygen.

Consequently, depending on the purity which is required, there are different ways to produce Oxygen.

On commercial scales, Oxygen obtains by the liquefaction and subsequent distillation of air. For very high purity Oxygen, it's necessary to take oxygen form air separation plant through a secondary distillation and purification stage. High purity Oxygen may be produced by the electrolysis of water. Lower purity of Oxygen can be produced with membrane techniques [1].

Several types of containers, depending upon the quantity, is used to store, ship, and handle liquid oxygen. Containers utilized are dewars, cryogenic liquid cylinders, and cryogenic storage tanks, and storage volumes vary from a few gallons to many thousands of gallons. Vaporization takes place continuously to maintain cold temperature. The vaporization rate varies depending on the design of the container and the volume of stored product. Containers are designed and manufactured according to the applicable codes and specifications for the temperatures and pressures involved [10].

Oxygen is shipped as a non-liquefied gas at pressures of 2000 psig (138 bar) or above inside the cylinders, also as a cryogenic liquid at pressures and temperatures below 200 psig (13.8 bar) and -232 0F (-146.5 0C) .

Whenever large quantities of gas are needed, the economical solution lies in the installation of a tank and evaporator system. The oxygen, nitrogen or argon is delivered in liquefied and refrigerated form by road tanker and then pumped into the customer's heat-insulated tank with the vehicle's pump.

A heat exchanger downstream of the tank evaporates the liquefied gas, which then flows through a pipeline to the user.

The size and type of storage vessel and vaporization is selected to suit customers' individual requirements.

The tanks consist of an inner vessel of cryogenic chromium-nickel steel to hold the liquefied gas and a supporting outer vessel of carbon steel. The space between the inner and outer vessels is airless and insulated with perlite.

Ambient air evaporators consist of aluminum tubes with longitudinal ribs. They work without auxiliary energy by heat exchange with the surrounding air. The liquefied gas is evaporated and heated to virtually ambient temperature.

The ambient air evaporators are modular in design and put together according to the desired capacity. For applications involving longer operating times, therefore, several evaporators are connected in groups. This allows one evaporator group to be used while the other is given time to regenerate.

The nominal capacities specified refer to eight hours continuous operation; thereafter the capacity of the evaporators can drop because of ice formation.

If the customer process requires gas, the liquid is vaporized and delivered as a gas along the supply pipe. If the process requires liquid, it is delivered directly from the storage vessel through a cryogenic vacuum insulated pipeline (Figure 1.1).

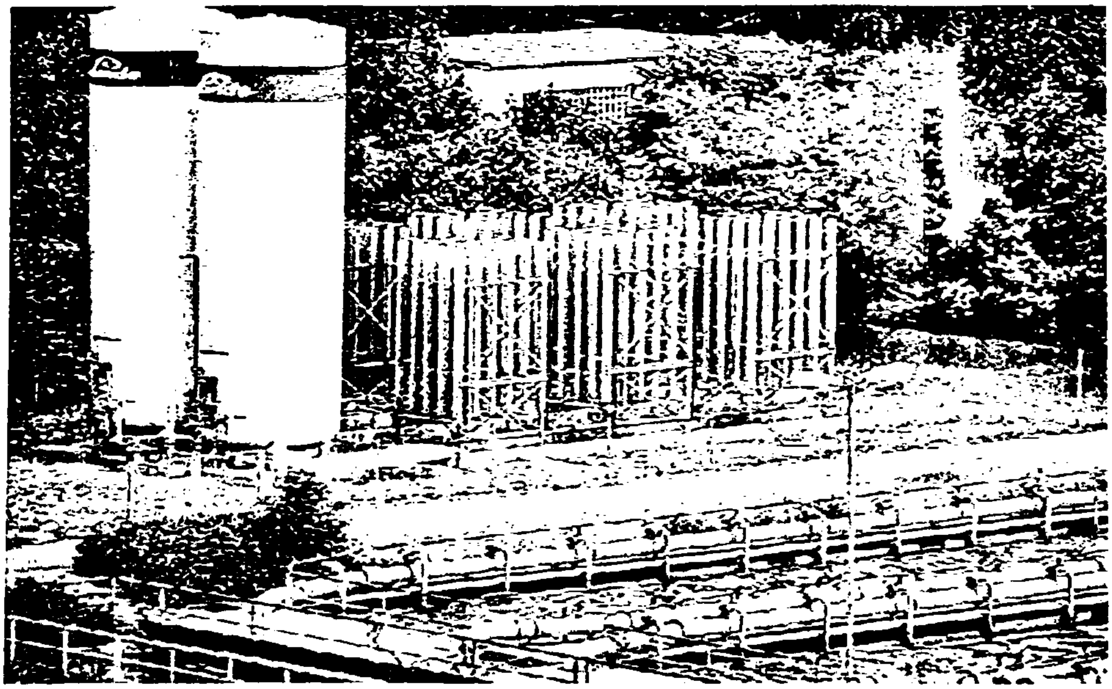

Figure 1.1: Cryogenic Storage tank and its related vaporizer.

All shipments of oxygen, refrigerated liquid (liquid oxygen), must comply with DOT regulations. This applies to motor freight, rail, air, and water shipments. Air shipment of oxygen, refrigerated liquid is forbidden. Water vessel shipments must also be prepared in accordance with the International Maritime Organization (IMO) regulations. All packaging used to transport oxygen must be either "UN/DOT Specification" or "UN/DOT Authorized" and in proper condition for transport. DOT Code of Federal Regulations,

Title 49, also specifies the following labeling and identification requirements:

DOT Shipping Name: Oxygen, Refrigerated Liquid

DOT Hazard Class: 2.2

DOT Shipping Label: Nonflammable Gas and

Oxidizer (For domestic shipments, only the oxygen label may be used) [10].

1.2: Nitrogen:

As follows some physicochemical characteristic of Nitrogen gas and Liquid Nitrogen is discussed. Nitrogen can be used in different segment of industry; some of these applications are noted in this chapter.

1.2.1: Atmospheric Nitrogen:

Nitrogen with symbol N and atomic number 7, is the primary gas in the atmosphere. As Nitrogen makes up 78.084% of the atmosphere by volume in dry air, it's the most common composition of the atmosphere.

Nitrogen is a colorless, odorless and tasteless gas, also its non-combustion element in the air [1].

In 1772, the Scottish physician Daniel Rutherford has been discovered the element Nitrogen as a separable component of air. In term of life on Earth, nitrogen is a vital element. It's a part of all plants and animal proteins. Nitrogen as a part of DNA and RNA molecules is an essential element of each individual's genetic scheme [13]. The human body contains about 3% by weight of Nitrogen. The forth most abundant element after Oxygen, Carbon and Hydrogen in human body is Nitrogen. Many compounds which are very important for industries, such as ammonia, nitric acid, organic nitrates (propellants and explosives), and cyanides, contain nitrogen. The extremely strong bond in elemental nitrogen dominates nitrogen chemistry, making difficulty for both organisms and industry in breaking the bond in order to convert the N_2 into applied compounds, but simultaneously causing release of huge amounts of often useful energy when the compounds burn, explode, or decay back into nitrogen gas [14]. The nitrogen cycle describes transformation of the Nitrogen from the air into the biosphere and organic compounds, then back into the atmosphere. This can be obtained in both biological and physical processes. The diagram below shows how different processes fit together in order to form the nitrogen cycle [15] (Figure 1.2).

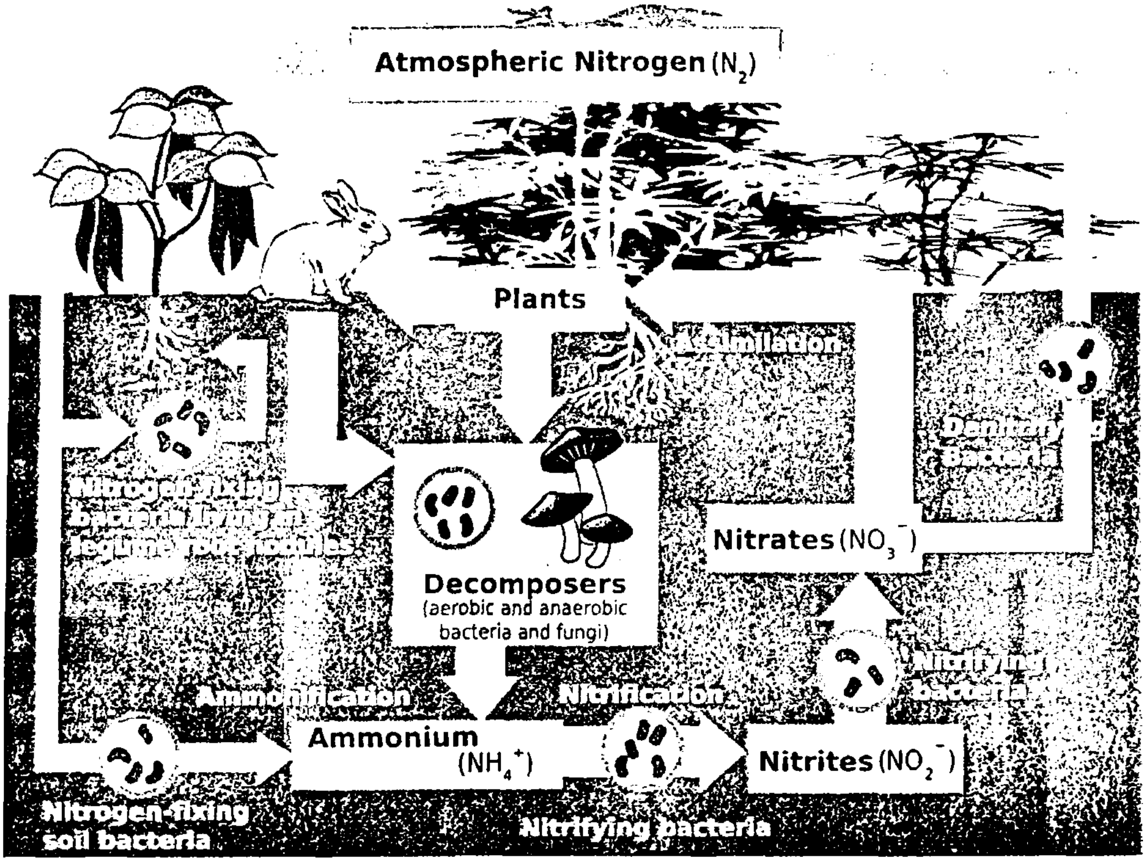

Figure 1.2: Nitrogen Cycle.

The most important processes in the nitrogen cycle are fixation, mineralization, nitrification, and denitrification. Nitrogen in the environment is present in a wide variety of chemical forms such as organic nitrogen, ammonium (NH_4^+), nitrite (NO_2^-), nitrate (NO_3^-), nitrous oxide (N_2O), nitric oxide (NO) or inorganic nitrogen gas (N_2). Organic nitrogen may be in the form of a living organism, humus or in the intermediate products of organic matter decomposition.

1.2.2: Liquid Nitrogen,

Liquid nitrogen is nitrogen in a liquid state with very low temperature (−195.8 °C). Liquid nitrogen is a colorless cryogenic liquid. It is often referred to by the abbreviation, LN_2 or "LIN" or "LN". Liquid nitrogen is a compact and suitable transported source of nitrogen gas.

Like all other gases, based on the purity which is required, there are different methods for producing Liquid Nitrogen. In air separation after liquefaction and subsequently air distillation a large amount of Nitrogen, Oxygen and usually Argon is produced. If very high pure Nitrogen is needed, the produced Nitrogen form air separation may need to go through a secondary purification process. Medium and high purities of Nitrogen are produced with Pressure Swing Absorption (PSA) techniques. The lower range of Nitrogen purities can be produced with membrane technique [1].

Liquid nitrogen at atmospheric pressure boils at −196 °C (77 K; −321 °F). Because it is very low temperature fluid careless handling of it may cause frostbite in contact with living tissues. When properly insulated from atmospheric temperature, liquid nitrogen can be stored and transported, for example in vacuum flasks. Here, the very low temperature is held constant at 77 K by very slow boiling of the liquid, resulting in the evolution of nitrogen gas. Depending on the size and design of the vacuum flasks, the holding time of Liquid Nitrogen is various from a few hours to a few weeks. Liquid nitrogen an easily be converted to the solid by placing it in a vacuum chamber pumped by a rotary vacuum pump [16]. Since the liquid to gas expansion ratio of nitrogen is 1:694 at 20 °C (68 °F), a huge amount of force can be produced if liquid nitrogen is quickly vaporized. Therefore, if Liquid Nitrogen won't be sealed properly, in other words if it'll be in contact with atmospheric temperature, the force generated by buildup pressure will be sufficient for a catastrophe and explosion. In an incident in 2006 at Texas A&M University, as a result of malfunctioning of Liquid Nitrogen storage tank a tremendous pressure buildup. The force of the explosion caused the tank to propel through the ceiling above it [17].

When liquid nitrogen evaporates it will decrease the amount of oxygen in the air and might causes asphyxia, especially in confined areas. Using Oxygen sensors when working with liquid nitrogen to alert workers of gas as a safety precaution is a good solution to prevent asphyxia [18]. All shipments of nitrogen, refrigerated liquid (liquid nitrogen), must comply with DOT regulations. This applies to motor freight, rail, air, and water shipments. Air shipment of nitrogen, refrigerated liquid is restricted depending upon amounts. Water vessel shipments must also be prepared in accordance with the International Maritime Organization (IMO) regulations. All packaging used to transport nitrogen must be either "UN/DOT Specification" or "UN/DOT Authorized" and in proper condition for transport. DOT Code of Federal Regulations, Title 49, also specifies the following labeling and identification requirements:

DOT Shipping Name: Nitrogen, Refrigerated Liquid

DOT Hazard Class: 2.2

DOT Shipping Label: Nonflammable Gas [19].

1.2.3: Application Of Nitrogen in Industry:

Nitrogen either as a gas or cryogenic liquid is used almost in every industry. In the chemical industry it's used for purging and blanketing. In semi-conductor industry, large quantities of high purity nitrogen are used as a pure or carrier gas as well as blanketing the equipment when they are not in production. In the electric industry nitrogen is used for inerting of epitaxial reactors. In automobile industry it's used to fill tires to lower wear as well as limit the risk of blow-outs. It is usually used as carrier gas in gas chromatography, or as a zero gas for analytical instrument. It's also used as a pure gas, or as a balance gas in different gas mixtures. In order to provide an oxygen free atmosphere during heat treatment of different metals nitrogen is used either pure or, more often, in a mixture with reducing gas such as natural gas or hydrogen. In food industry nitrogen is used both in gas and liquid phases. In mixtures with Carbon dioxide, nitrogen is used for packing food stuff in order to increase the food shelf - life and decrease the risk of food decay. Liquid nitrogen is used to freeze a vast variety of delicate food such as strawberries, fish and shrimps, hamburgers etc. Also it is used in food preparation, such as for making ultra-smooth ice cream.

In the nuclear industry, liquid nitrogen is used for scientific researches. Liquid nitrogen is used extensively to cryopreservation of biological materials and samples such as tissue, cells commonly reproductive cells (sperm and egg). Liquid nitrogen is used also for cryo surgery to remove cysts and warts on the skin [1].

Like dry ice, the main application of liquid nitrogen is as a refrigerant. It is used in cold traps for certain laboratory equipment and to cool infrared

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Like dry ice, the main application of liquid nitrogen is as a refrigerant. It is used in cold traps for certain laboratory equipment and to cool infrared detectors or X-ray detectors. It has also been used to cool central processing units and other devices in computers that are overclocked, and that produce more heat than during normal operation.

1.2.4: Supplying and storing Industrial Gases (N2 and O2):

As mentioned before Industrial gases are used in a vast range of manufacturing processes and the requirement of the individual customer is various depending on their demands. The key factors which must be considered when determining the most suitable and cost effective gas supply mode for each customer are Pressure, volume and flow rate. Generally, Gases are supplied in different forms from cylinders either as a high pressure gas or as a low pressure liquid. These cylinders have a limited volume of gas but they are easy to handle and move to where they are required. As Oxygen and Nitrogen are liquefied at low temperature can be supplied either in small cryogenic vessels or in large tanks capable of holding tens of thousands of liters' of liquid gas. Gases such as oxygen, nitrogen, argon, hydrogen and helium can be easily compressed into a cylinder at pressures up to 300 bar. These cylinder's materials are capable of withstanding these pressures.

Traditionally these cylinders have been constructed by high strength steel or aluminum but nowadays modern composite cylinders are now becoming available. Each cylinder is fitted with a cylinder valve which is compatible with the gas and pressure requirements. The outlet thread is dictated by international standards to ensure that merely regulators compatible with these requirements can be fitted (Figure 1.3).

A cryogenic liquid cylinder is an insulated, vacuum-jacketed, pressure vessel. They are equipped with pressure-relief valves and rupture disks to protect the

cylinders from pressure buildup. Liquid containers operate at pressures up t
350 psig and have capacities between 80 and 450 liters of liquid [10].

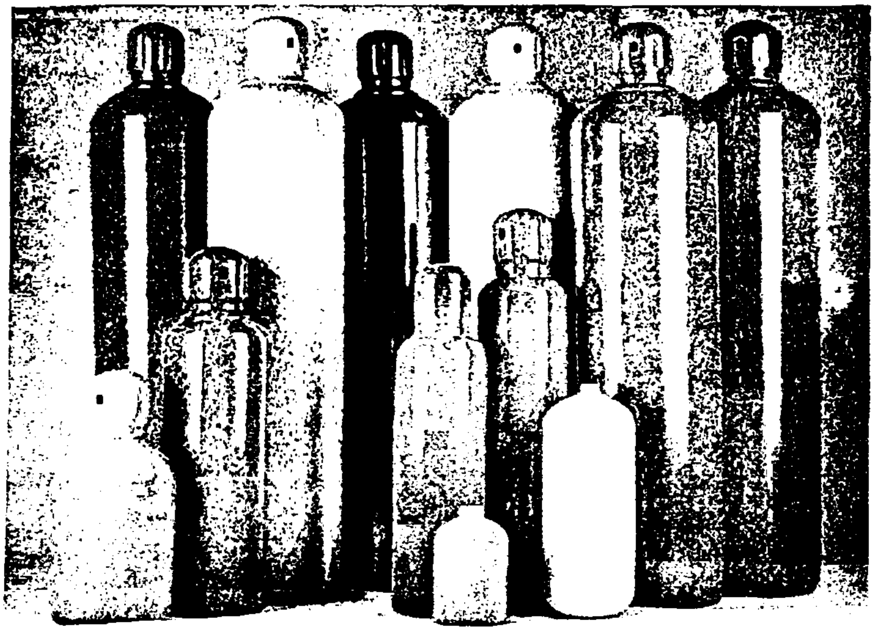

Figure 1.3: Gas Cylinders.

Bundles - which are multiple cylinders connected together - is another solutio for applications requiring higher capacities of cylinders (Figure 1.4).

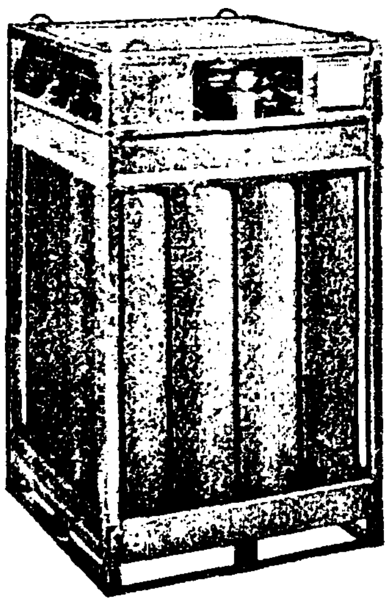

Figure 1.4: Gas Bundle.

A loose-fitting dust cap over the outlet of the neck tubes prevents atmospheric moisture from plugging the neck and allows gas produced from vaporized liquid to escape. This type of container is nonpressurized [10].

Drum tanks – Dewar - which are an averaged sized supply vessel can commonly provide between 400kg and 1500kg of gas contents. Drum tanks is suggested for the customers whose demand is too high to be simply managed via cylinder supply, but not high enough to be rational for delivery in bulk/ISO tanks. On the other hand, the advantage of drum tanks is minimizing the need for cylinder handling, such as in changeovers and site transport, as well as reducing space requirements (Figure 1.5).

Cryogenic liquid cylinders which are pressurized vessels are sometimes incorrectly referred to as dewars.

Figure 1.5: Dewar.

Whenever large volumes of gas are needed, the economical solution is the installation of a tank and evaporator system. Large quantities of gas are supplied by bulk deliveries, either as a cryogenic liquid or a high pressure gas into storage on customer sites. The oxygen, nitrogen or argon is delivered in liquefied and refrigerated form by road tanker and then pumped into the customer's storgae tank with the vehicle's pump. Oxygen and nitrogen are supplied as liquid because it requires much less storage capacity than gas.

The liquid is delivered by the dedicated fleet of cryogenic tankers into vacuum insulated bulk storage vessels. The stored liquid is controlled at the required pressure by means of an automated regulation system (Figure 1.6).

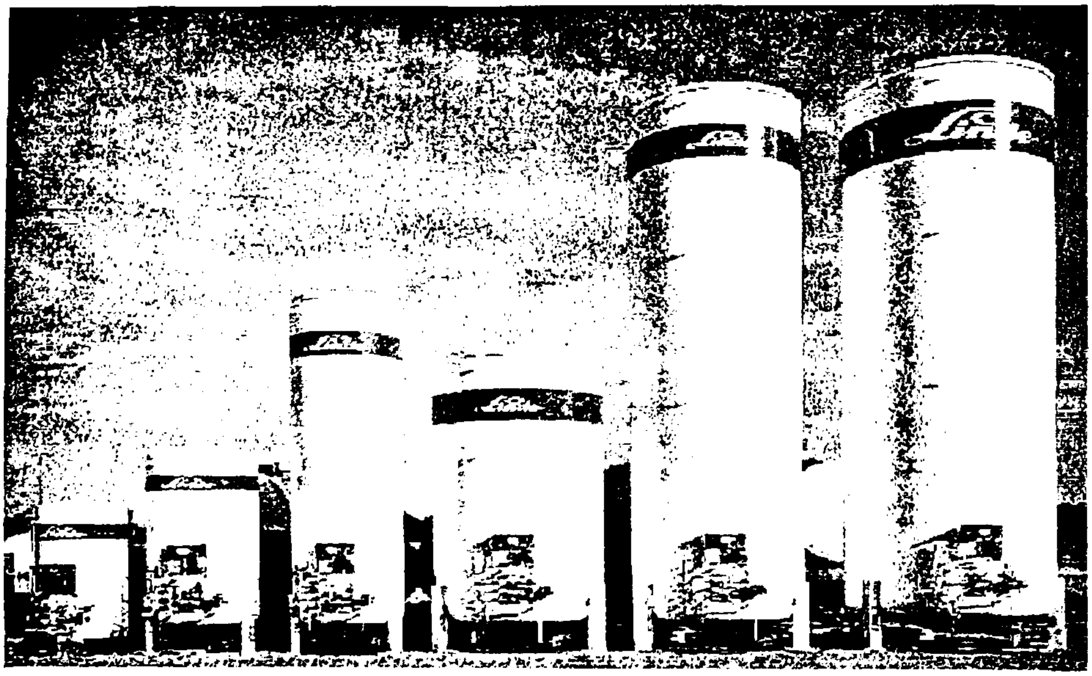

Figure 1.6: Cryogenic storage Tank.

If the customer process requires gas, the liquid is vaporized and delivered as a gas along the supply pipe. . Used in parallel with cryo tanks, vaporizers convert liquid into gas. If the process requires liquid, it is delivered directly from the storage vessel through a cryogenic vacuum insulated pipeline. The size and type of storage vessel and vaporization is selected to suit customers' individual requirements Storage vessels and high pressure gas tube trailers can be fitted with telemetry systems to provide real time remote monitoring of stock levels ensuring product is always available.

For customers requiring even larger quantities of Oxygen and Nitrogen there is another cost efficient solution which is On-site supply systems. Both atmospheric and chemical gases can be supplied with On-site system with the various demanded of quantities and qualities.

CHAPTER 2: NEED OF INDUSTRIAL GASES:

Oxygen and Nitrogen are two undeniable factors in biosecurity of human being and animal life. Nitrogen is playing a very important role in food industry especially in packing segment. Oxygen is also essential in all period a creature life period.

In this chapter after a brief introduction of aquaculture and food industry, the effect of Oxygen and Nitrogen is discussed to show how they can be helpful to improve these industries.

2.1: Animal Breeding:

To breed animal, especially different type of fishes Oxygen is the most essential factor. The optimum growth and healthy animals need a specific amount of oxygen in his environment. The more or the less amount of needed oxygen has harmful effect on the life style of that specific species or even may cause death. In the following segments the effect of oxygen in fish breeding has been described more specifically.

2.1.1: Aquaculture Industry:

As defined by the United Nations Food and Agriculture Organization (FAO), aquaculture which also known as aquafarming is the "farming of aquatic organisms including fish, molluscs, crustaceans and aquatic plants. Farming implies some sort of intervention in the rearing process to enhance production, such as regular stocking, feeding, protection from predators, etc. Farming also implies individual or corporate ownership of the stock being cultivated…" [20].

In other word Aquaculture is a form of agriculture that involves the propagation, cultivation, and marketing of aquatic animals and plants in a controlled environment [21].

World fish farming was first practiced as long ago as 2000 B.C., in China. Ornamental fish ponds appear in paintings from ancient Egypt. European aquaculture began sometime in the middle Ages and transformed the "art" of Asian aquaculture into a science that studied spawning, pathology, and food webs. One of the most significant developments was the invention of culture methods for trout, which were being introduced into natural waters by the mid-1800s.

Nowadays, the vast majority of aquaculture takes place in Asia. In 2002, over 70% of worldwide aquaculture production was in China alone [20].

To meet rising demand for seafood worldwide, more and more fish have to be raised in fish farms. Aquaculture is an essential link in the agricultural chain asit protects the oceans from further over-fishing and produces healthy food with extremely high feed conversion efficiency [22].

With a growth rate of 8% per year since the 1980's, aquaculture is probably the fastest growing food-production industry, that today accounts for almost half the fish consumed globally, up from 9% in 1980 [23].

Aquaculture offers a solution to meet the growing demand for seafood that catching fish cannot provide. Fish farming now accounts for roughly one-third of the world's total supply of food fish and undoubtedly the contribution of aquaculture to seafood supplies will increase in the future. Aquaculture has the potential to become a sustainable practice that can supplement capture fisheries and significantly contribute to feeding the world's growing population.

A large proportion of organisms that humans rely on for protein and sustenance come from the sea. Currently, approximately 16 percent of animal protein consumed by the world's population is derived from fish, and over one billion people worldwide depend on fish as their main source of animal protein.

Worldwide consumption of fish as food has risen from 40 million tons in 1970 to 86 million tons in 1998 [24].

Once thought of as an abundant, inexhaustible resource, the world ocean faces a significant loss of essential diversity. This loss is occurring at an alarmingly rapid rate, due to the combined effects of overfishing, habitat destruction, pollution, and profound ecological and biotic change caused by global warming as well as the human-mediated transfer of marine organisms.

According to the FAO "About 47 percent of the main stocks or species groups are fully exploited and are therefore producing catches that have reached, or are very close to, their maximum sustainable limits [20].

Clearly, additional means of producing fish must be developed in order to maintain a sufficient supply of food for an ever-growing population. Aquaculture offers one way to supplement the production of wild capture fisheries and it will continue to increase in importance as demand increases in the future.

It was not until after World War II that aquaculture gained much attention as a potentially large scale industry. A shift in economic conditions in developed nations of the world led to an increase in the demand for fish such as salmon, shrimp, eels, and sea basses, all of which can be produced profitably through aquaculture [25].

In the 1960's, aquaculture became a significant commercial practice in Asia where it had mainly been used as a small-scale means of local community food production for thousands of years. In the last few decades, worldwide aquaculture production has increased significantly. In 1970 aquaculture operations composed 3.9 percent of all fish production, compared to 27.3 percent in 2000. Worldwide, total fish production from aquaculture operations has increased steadily at a rate of 9.2 percent per year.

But, aquaculture has not yet become the large-scale global food replacement for the numerous food-poor areas of the world, as many thought it would be [20].

Fisheries and aquaculture make crucial contributions to the world's wellbeing and prosperity. In the last five decades, world fish food supply has outpaced global population growth, and today fish constitutes an important source of nutritious food and animal protein for much of the world's population.

In addition, the sector provides livelihoods and income, both directly and indirectly, for a significant share of the world's population.

Fish and fishery products are among the most traded food commodities worldwide, with trade volumes and values reaching new highs in 2011 and expected to carry on rising, with developing countries continuing to account for the bulk of world exports. While capture fisheries production remains stable, aquaculture production keeps on expanding. Aquaculture is set to remain one of the fastest-growing animal food-producing sectors and, in the next decade, total production from both capture and aquaculture will exceed that of beef, pork or Poultry [26].

Aquaculture can be more productive and profitable than land-based agriculture. Here, there are some reasons which show the advantages of aquaculture in compare with land-based agriculture.

Meaningful comparisons of productivity are complicated, but fish have certain advantages over land animals in their suitability for farming. Being cold-blooded, fish do not have to expend energy in maintaining body temperature. Also, unlike land animals, they do not have to support their weight and should therefore be inherently more efficient at converting food into flesh. Because a fish farm uses a three-dimensional rearing area, fish have the added dimension of depth in which to grow, thus increasing yields on a per acre basis. Production in ponds can approach 10,000 pounds per acre annually compared to approximately 1,000 pounds per acre annually for beef cattle. In general, fish have a lower proportion of inedible bones and offal, which means a greater processed weight for the producer.

Many of these early attempts at fish husbandry failed because operators were not experienced in fish culture, ponds were not properly built, low-value species were being raised, and finally selected species lacked adequate technical support [21].

Many have looked to aquaculture to fill in the gaps left by dwindling fisheries. Undoubtedly, this is a genuine possibility. Its realization, however, will depend on how the aquaculture industry develops in the future. At this point in time, the development of aquaculture is at a crossroads.

Unsustainable development will only generate short and medium-term profits for multinational corporations at the expense of long-term ecological balance and social stability. More sustainable development alternatives are needed to ensure that in the future aquaculture can contribute to the growing need for seafood products. There are a number of alternative ways forward in the development of aquaculture, which can offer more sustainable solutions. In some cases these methods have been around for centuries, but they have rarely been adopted in the modern aquaculture industry, and in other cases they are innovative practices that can be explored by aquaculture proponents. Alternatives include ecological aquaculture, organic aquaculture, polyculture, mollusc farming, and closed and low discharge systems.

These alternative practices have been successfully implemented in different areas of the world. However, they must be examined for their application on a wider scale. While each of the mentioned potentially sustainable practices does have some environmental impacts, they can be greatly minimized if the systems are managed well. In addition to environmental considerations, social and economic aspects must be considered when assessing each of these practices [20].

2.1.2.Overview of Aquaculture Industry:

A number of aquaculture practices are used world-wide in three types of environment (freshwater, brackishwater, and marine) for a great variety of culture organisms. Freshwater aquaculture is carried out either in fish ponds, fish pens, fish cages or, on a limited scale, in rice paddies. Brackish water aquaculture is done mainly in fish ponds located in coastal areas. Marine culture employs either fish cages or substrates for molluscs and seaweeds such as stakes, ropes, and rafts [27].

Farming systems are diverse for example including:

- Water-based systems (cages and pens, inshore/offshore).
- Land-based systems (rainfed ponds, irrigated or flow-through systems, tanks and raceways).
- Recycling systems (high control enclosed systems, more open pond based recirculation).
- Integrated farming systems (e.g. livestock-fish, agriculture and fish dual use aquaculture and irrigation ponds).

The phases of aquaculture include broodstock holding, hatchery production of seed, nursing systems, grow-out systems, and quarantining.

Together, this mix of intensity, culture systems, species, farming systems and different phase of culture create an extreme diverse collection of aquaculture systems and technologies [28].

A high degree of technological flexibility makes aquaculture feasible under a variety of conditions and objectives. Aquaculture may be practiced at different intensity levels. Simple systems requiring low levels of technological management and resources, and only slight modifications of the environment are termed "extensive." Aquaculture becomes increasingly "intensive" as more control of the environment and sophistication in management are used. An important aspect of aquacultural technology is the use of nutrient inputs in the form of fertilizers, foods or both. Extensive aquaculture uses low- quality foods and fertilizers in small amounts. Higher quality inputs in large amounts are required for intensive aquaculture.Small-scale aquaculture for the promotion of socio-economic development fulfills the objectives of food production, income generation and provision of local employment for small farmers. Extensive technology and associated low operating costs with higher labor requirements are often mandated by the reduced availability of investment and operating capital for small-scale farmers. Large-scale or "industrial" aquaculture is more concerned with maximizing profit through sales and relies on more intensive technology. Larger capital outlay and more advanced management skills are required.

Currently, more than 220 species of finfish and shellfish and dozens of aquatic plant species are cultured in a variety of freshwater, brackish and marine environments [29].

2.1.3.Essential Factors in Aquaculture:

Water supply and quality are the most important factors in selecting the proper location for an aquaculture facility. Water quality includes all physical, chemical and biological factors that influence the beneficial uses of water. Where aquaculture is concerned, any characteristic of water the affects the survival, production, growth or management of fish or other aquatic creature in any way is a water quality variable. There are many water quality variables in ponds aquaculture but a few of them are normally playing an important role. These are the variable which aqua culturist should attempt to control by management techniques [30].

The success of a commercial aquaculture enterprise depends on providing the optimum environment for rapid growth at the minimum cost of resources and capital. Quality of water is, therefore, an essential factor to be considered when planning for high aquaculture production [31].

Wells and springs are the best sources of water, but other sources are acceptable if the quality and quantity are adequate. Important water quality characteristics to consider are temperature, dissolved oxygen, ammonia, nitrites, nitrates, pH, alkalinity, and hardness.

Among the different types of aquaculture, the production of food organisms such as catfish, trout, and hybrid striped bass are the best known.

Organisms in each of type aquaculture will be produced using one of the following methods: ponds, cages, raceways, or water-recirculating systems.

As follows some of the basic concepts for the husbandry of aquatic organisms, specifically fish, will be discussed [21].

2.1.3.a.Water Sources:

The most important factor in selecting the proper location for an quaculture facility is water supply and its quality. Aquatic organisms depend upon water for all their needs. Fish need water in which to breathe, eat, grow, and reproduce. Large quantities of water must be available year-round. If water is not available all the time, but there is some way to store it, then that site still may be suitable. The key, of course, is that water must always be available and in good supply [21].

Water quality is determined by the substances dissolved in it. Water of good quality has an adequate balance of chemicals for efficient growth of healthy fishes. Three factors determined water quality in ponds: The original source of the water, the chemical nature of the soil and finally substances added to the water once it is in the pond [32].

Water sources can be classified as: wells, springs, groundwater, streams, rivers, lakes, and municipal.

Of these seven possible sources, wells and springs rank the highest in terms of overall quality. Wells and springs are usually uncontaminated and have no unwanted fish or fish eggs. The only drawbacks to well and spring water are their low concentrations of dissolved oxygen (which fish need to breathe), and their high concentrations of dissolved gases such as carbon dioxide, and metals such as iron. These problems can be overcome. Groundwater sometimes is used where ponds are dug into the existing water table. This type of pond is generally less productive than ponds filled from other sources because of the low productivity of the surrounding soil.

Streams, rivers, and lakes also can be used to produce aquatic organisms, but they are subject to any contaminants that could wash in from the surrounding watershed. In addition, unwanted fish or fish eggs must be filtered from these existing water bodies.

To great extent water quality determines the success or failure of a fish farming operation. Physical and chemical characteristics such as suspended solids, temperature, dissolved gases; pH, mineral content, and the potential danger of toxic metals must be considered in the selection of a suitable water source. However, dissolved oxygen is the most important and critical parameter, requiring continuous monitoring in aquaculture production systems. This is due to fact that fish aerobic metabolism requires dissolved oxygen [33].

Of these many water quality characteristics, only temperature, dissolved oxygen, ammonia, pH, and alkalinity will be discussed, and among these variable factors we mainly focus on dissolved oxygen.

2.1.3.b. Temperature:

Energy from the sun and air temperature are the two main factors that influence water temperature. But there are other influences, as well. Inflows and outflows (creeks, streams, wastewater discharge, groundwater seepage, etc.), the shape and depth of the lake basin (i.e., lake morphometry); wind and waves; even the color of the water can influence temperature [34].

As mentioned, fish are cold-blooded organisms and assume approximately the same temperature as their surroundings. Metabolic rates increase rapidly as temperatures go up. Many biological activities such as spawning and egg hatching are geared to annual temperature changes in the natural environment. These temperatures vary according to the particular species. Fish are generally categorized into warmwater, coolwater, and coldwater based on optimal growth temperatures [21].

Each species of fish has its own preferred temperature range. Within that range, fish will grow faster of higher temperatures [32].

Channel catfish are an example of a warm water species, with a temperature range for growth between 70° and 85°F. A temperature of 82°F is generally considered optimum for growth.

Striped bass, hybrid striped are examples of coolwater species. Ranges for Optimum growth fall between 55° and 75°F. Temperatures in the upper end of this range are generally considered best for maximum growth for all coolwater species.

Coldwater species include all species of salmon and trout. Two of the more commonly cultured coldwater species are rainbow trout, and to a lesser extent, brown trout. Their optimal temperature range for growth is 48°–60°F.

Ideally, species selection should be based partly on temperatures of the water supply. Any attempt to match the fish with improper water temperatures will involve energy expenditures for heating or cooling to within the desired range. This added expense will subsequently reduce the farmer's profits [21].

Extreme fluctuation in water temperature always stress fishes and leads to reduce growth and possibility even sickness.

Water temperature plays the largest role in influencing the amount of oxygen in a water body. The oxygen holding capacity of water decreases as the water temperature rises. The rule of thumb: warm water holds less oxygen than cool water [34].

2.1.3.c. Nitrogen & Ammonia:

Nitrogen may enter ponds from atmosphere in molecular form (N_2), and some molecular Nitrogen can fix in organic compounds by blue-green algae and bacteria.

Rain falling into ponds contains nitrate and various form of nitrogen may enter ponds via the water supply. Inorganic nitrogen may be added in fertilizer and organic nitrogen in manure or feed [30].

Fish excrete ammonia and a lesser amount of urea into the water as wastes. Two forms of ammonia occur in aquaculture systems: ionized (NH_4^+) and un-ionized (NH_3). The un-ionized form of ammonia is extremely toxic to fish and causes "brown blood" disease. Ionized ammonia is not. Both forms are grouped together as "total ammonia nitrogen." Through biological processes, toxic ammonia can be degraded to harmless nitrates as shown below:

$$\text{Ammonia } (NH_3) \xrightarrow[\text{bacteria}]{O_2} \text{Nitrites } (NO_2^-) \xrightarrow[\text{bacteria}]{O_2} \text{Nitrates } (NO_3^-)$$

In natural waters, such as lakes, ammonia may never reach critically high levels due to the low densities of fish. When fish are cultured intensively and fed protein-rich feeds they can produce high concentrations of ammonia in the water. Analytical methods are used to determine total ammonia-nitrogen (TAN). The proportion of TAN that exists in ionized and un-ionized form varies with pH and temperature. As pH and temperature increase, the amount of TAN in the toxic un-ionized form increases [35].

Toxicity levels for un-ionized ammonia depend on individual species; however, levels below 0.02 ppm are generally considered safe. Ammonia and other metabolic wastes are gradually removed by natural processes in ponds or through the use of biological filters in recirculating and reuse systems, where water is continually recycled. However, the intermediate form of ammonia – nitrite – has been known to occur at toxic levels in fish ponds [21]. Concentrations of 0.5 ppm have reduced growth and adversely affected fish. Fish can tolerate nitrate to several hundred ppm. Removal or detoxification of ammonia is facilitated by providing and maintaining an optimal environment for the appropriate bacteria (pH between 7-9; temperature approximately 75-85° F) [35].

2.1.3.d. PH:

The quantity of hydrogen ions in water determines whether it is acidic or basic. The scale for measuring the degree of acidity is called the Ph scale, which ranges from 1 to 14. A value of 7 is neutral, neither acidic nor basic; values below 7 are considered acidic; above 7 basic.

All living organisms have optimal ranges of pH where growth is best. The acceptable pH range for fish culture is normally between pH 6.5 and 9.0 [21]. If pH readings are outside this range, fish growth is reduced. At values below 4.5 or above 10, mortalities occur.

Soil and water pH is easily tested and low pH is regulated by the addition of limestone. Since pH fluctuates naturally during the day, be consistent in the time of measurement to be able to better compare changes in pH from day to day. The consumption of carbon dioxide causes pH to naturally fluctuate during the day. It is generally lowest at sunrise (due to accumulation of carbon dioxide during the night) and highest at afternoon when algae consumption of carbon dioxide is at its greatest. Waters of moderate alkalinity are more buffered and the degree of pH fluctuation is lower [35].

2.1.3.e. Alkalinity & Hardness:

Alkalinity is an important water quality parameter. Total alkalinity is defined as the total concentration of bases in water as expressed in mg/L of equivalent calcium carbonate ($CaCO_3$). The most abundant bases found in water include hydroxide (OH^-), bicarbonate (HCO_3^-), and carbonate (CO_3^{2-}). These bases originate from the dissolution of limestone in soils [36].

Alkalinity can affect the potential for primary productivity and also the water pH. Values of 50-100 mg/l are considered moderate and are recommended.

Addition of limestone can increase the concentration of carbonates and alkalinity. Alkalinity in ponds needs to be measured once a month [35].

It plays a couple of important roles in water. Bicarbonates and carbonates, as well as carbon dioxide, are a source of carbon used for photosynthesis. Bicarbonates and carbonates are also a major part of the buffering system, which curtails large daily pH fluctuations. The natural productivity of pond systems increase when alkalinity levels are higher than 20 mg/L. Adding lime can raise alkalinity in earthen ponds [36].

Hardness is the measure of the calcium and magnesium portion of the buffering system. These two elements can be absorbed by the fish's gills and, in addition to other uses , they help with the bone development in fish. Fish will grow over wide ranges of alkalinity and hardness, but values of 120–400 ppm are considered optimum [21].

2.1.3.f. Dissolved Oxygen:

Dissolved oxygen is one of several important water quality parameters that a producer will monitor daily. Maintaining good dissolved oxygen concentrations is essential for successful production [37].

Water — H_2O — is a simple molecule made up of two atoms of hydrogen (H_2) and one atom of oxygen (O). However, the oxygen that fish and other organisms use underwater does not come from the actual water molecules themselves. That's because the single atoms of oxygen found in water molecules are bound to the two hydrogen atoms and are not available. Instead, all aquatic organisms use dissolved oxygen gas (O_2) for respiration.

Temperature and altitude are the two main factors that set the limits on just how much Oxygen can be held by water [34].

Dissolved oxygen gas (O2) is constantly entering water from two main sources: the atmosphere and from photosynthesis. Oxygen from the atmosphere continuously enters the surface of a water body through a process known as diffusion. Molecule by molecule, oxygen gas (02) is pushed into the water by pressure from the air above. Wind and wave action can accelerate the diffusion process because waves create more surface area for oxygen to enter the water. Artificial wave action, via aerators, can also increase oxygen concentrations in water.

Photosynthesis is perhaps the most critical source of oxygen, especially in water bodies where algae or aquatic plants are abundant. Oxygen is a by-product of this activity. As long as photosynthesis is taking place, oxygen is continuously being released into the water. In the early morning hours, or in the evening, or during low light conditions, photosynthetic activity is reduced. At night, it stops all together. The amount of oxygen in a water body is constantly changing. This is due to the fact that, even as oxygen is entering the aquatic environment, it is also being removed by biological activity within the water.

Biological activity includes the regular day-to-day functions of virtually all the inhabitants of a water body, including algae, bacteria, fish, insects, plants, etc. As these organisms carry on about their normal activities, they are constantly removing oxygen from the water and releasing carbon dioxide as a by-product. This process is known as respiration. Respiration is essentially the opposite of photosynthesis. Much of the time, the respiration that occurs within a water body is offset by photosynthesis so there is a surplus of oxygen available in the water.

Photosynthetic activity is reduced under low light conditions (e.g., cloudy weather). This means that once the sun goes down, algae and aquatic plants are no longer producing oxygen but they are continuing to consume oxygen. As a result, the water body's oxygen supply takes a double hit." If a lake experiences several days of low oxygen production due to cloudy weather or other low light conditions, it could encounter low oxygen concentrations that can be detrimental to fish and other organisms in the water [34].

Dissolved oxygen (DO) concentrations are expressed in parts per million (ppm) or milligrams per liter (mg/l). Both methods are the same since 1 mg/l is equal to 1 ppm. Some fish such as tilapia and carp are better adapted to withstand periodic low DO concentrations [21].

In most freshwater environments, the "normal" dissolved oxygen concentration (DO) measurements usually range somewhere between six and ten milligrams per liter (mg/L). When measurements drop down to three or four milligrams per liter, fish and other aquatic life will begin to experience stress, especially if the drop in oxygen occurs suddenly. Few organisms are able to survive in water when dissolved oxygen levels are below 2 milligrams per liter [34].

The oxygen that fish need to breathe also is consumed by the breakdown of fish wastes and uneaten feed [21].

The recommended minimum dissolved oxygen requirements are as follows: Cold water fish - 6 mg per litre (70% saturation) ,Tropical freshwater fish- 5 mg per litre (80% saturation) ,Tropical marine fish- 5 mg per litre (75% saturation) [31].

Overall health and physiological conditions are best if the dissolved oxygen is kept closer to saturation. When the levels are lower than those mentioned above, the growth of the fish can be highly affected by an increase in stress, tissue hypoxia, and a decrease in swimming activities and reduction in immunity to diseases. However, there is a need to maintain the level of dissolved oxygen at the saturation level which will not affect its physiological or metabolic activities, so as to have high production in any culture system [38]. More than that, one has to keep in mind that the oxygen level requirement depends on the species, but also on fish size and activity of the fish.

Oxygen is important in respiration and metabolism processes in any animal. In fish, the metabolic rate is highly affected by the concentration of oxygen in the rearing environment. As the dissolved oxygen concentration decreases, respiration and feeding activities also decrease. As a result, the growth rate is reduced and the possibility of a disease attack is increased. However, fish is not able to assimilate the food consumed when DO is low [39].

2.1.4. Oxygen Solubility in Water:

As mentioned before, the source of dissolved oxygen (D.O.) in natural waters is from atmosphere and photosynthesis of plants. Oxygen is absorbed in water by direct diffusion and by surface-water agitation. Solubility of oxygen in water is so small and by diffusion process alone in still water. It was calculated that it would take 6 years for oxygen to diffuse from the surface to a depth of 6 metres in quiet water. Absorption of water is very minor, that almost all the oxygen enrichment of natural waters takes place by agitation of water.

Photosynthesis of plants supply oxygen considerably - but this is limited to the zones to which light penetration takes place. Photoperiod (day light duration), diurnal and seasonal, would decide the amount of oxygen produced by photosynthesis. Daily pulses of oxygen could be seen clearly in waters with high concentration of plankton - with an afternoon maximum and early morning drop, in some cases taking D.O values close to zero. This can be seen in the tropical waters, where phytoplankton blooms occur [40].

Oxygen as a gas has a low solubility in water. In addition, the amount of oxygen contained in water varies with temperature and salinity in a predictable manner. Less oxygen can be held in fully air-saturated warm sea water than fully air-saturated cold freshwater [31].

Do content in water reducing sharply with increase in temperature. It becomes obvious from the values of solubility of oxygen that the warm tropical and semitropical waters have ordinarily contain much less oxygen than those of the temperate, but fish production in the tropics does not appear to be limited by this relative lesser availability of oxygen; as we know the rate functions which enhance growth are higher at the warmer tropical temperature (unless it is too high) and generally the tropical fishes have a better capacity to extract oxygen from oxygen deficient waters, by means of adaptations of hemoglobin. We shall be reverting to this when species relations are considered.

Solubility of oxygen in water changes markedly with depth of a water body, increasing with the increasing pressure with depth.

Solubility of any gas decreases markedly with increase in dissolved solids. This is not obvious for DO in ordinary freshwater, but is marked when the salinity increases. The D.O value changes from 7.6 in freshwater (0 ppt.S.) to 6.8 at (27 ppt.S.) and 6.4 in sea water (35 ppt.S.). This reduction in oxygen availability should be considered, when aquaculture practices are planned in brackish water and sea water conditions.

While the oxygen content of the water sets the absolute availability of oxygen in the water, it is the oxygen partial pressure gradient that determines how rapidly oxygen can move from the water into the fish's blood to support its metabolic rate. This is because oxygen moves by diffusion across the gills of fish. According to Fick's law of diffusion, the rate of diffusion of oxygen across the gills is determined by the gill area, the diffusion distance across the gill epithelia, the diffusion constant and the difference in partial pressure of oxygen across the gills [41].

In view of the importance of gas content (in solution) of a mass of water we shall recall the gas laws, which we all must be familiar with: Henry's law, which states that “the concentration of saturated solution of a gas is proportional to the pressure at which the gas is supplied and Dalton's law of partial pressure, which states that, “the pressure exercised by a gas is proportional to the component to its concentration in the mixture, and the total pressure of the gas is equal to sum of its component”.

Thus the amount of constituent gases in atmosphere that will dissolve in water depends on the proportion of each gas in the air and the solubility of each constituent gas. As it’s shown in Table.1.1.1, the volume of atmospheric air contain 78.084% nitrogen, 20.946% oxygen, and 0.934% argon. The remaining 0.036 % contains carbon dioxide, noble gases and trace gases such as helium, krypton, neon and xenon plus water vapor.

The partial pressure in the liauid phase that would be in equilibrium with the measured concentration is called the dissolved gas pressure (TGP). Under natural conditions any of the three states can occur in water:

BP (water is supersaturated - BP Barometer pressure in mm) = TGP

BP (in equilibrium) = TGP

BP (water is undersaturated) = TGP

Percentage saturation can be indicated in terms of the constituent gases, or in terms of the total dissolved gases:

$$TGP\ (\%\ saturation) = \frac{100 \times TGP}{BP}$$

At an atmospheric pressure of 760 mm Hg (NTP) the partial pressure of oxygen (PO_2) will be 159 mm Hg i.e.

$$\frac{760 \times 20.946}{100}$$

At 100% air saturation solubility of oxygen at 30°C is 7.54 mg/1 [40].

Consequently, partial pressure of oxygen is the most appropriate term for expressing oxygen levels in aquaculture water. However, oxygen concentration is the more commonly used term and, for a given temperature and salinity, the partial pressure of oxygen and oxygen content in water are linearly related. Another suitable method for expressing oxygen levels in aquaculture is % air saturation (often reduced to just % saturation) which is directly proportional to the partial pressure and is reported on most oxygen probes that have built in algorithms for temperature and salinity [42].

Since finfish and shellfish depend on primarily oxygen dissolved in water for sustenance (the air-breathing fishes are exceptions, but their capacity for tolerance of hypoxic conditions can be made use of in aquaculture e.g. fish culture in sewage oxidation ponds) the relative handicaps in having water as respiratory medium as compared with air can be pointed out.

Since the content of oxygen in water is very much less in water than in air, the fish has to pump through its gills relatively a larger amount of the medium, which is much heavier too, to extract sufficient oxygen to sustain itself. An air-breather in this connection has relatively a greater saving of energy and it is yet to be established if the air-breathing fish fares better in energy conversion and growth because of this advantage but the air-breathing fish spends considerable energy in surfacing frequently especially if it is in deep water. It is interesting to explore this aspect further. Water appears to be a risky medium when compared with air and the variety of adaptations of aquatic life to ambient oxygen bear testimony to this.

The low diffusion of oxygen in water referred to already and the stratification of gases even in small bodies of water, unlike atmospheric air which is uniform, make each water body distinct in its nature. This has significance in that each water body has to be therefore carefully studied for choosing it for aquaculture [40].

2.1.5.Oxygen Application In Aquaculture:

As it's mentioned above, dissolved oxygen is the most essential factors in water quality for a successful aquaculture, and requiring continues monitoring in a production system [31].

The current trend in intensive culture systems is to aim for a high stocking density at low water consumption per kilogram of biomass. This cannot be achieved without oxygen. Through oxygenation of the water, fish and seafood farmers can protect their stock against oxygen deficiencies during peak consumption and growth periods. Adequate oxygen levels in the water at all times not only ensure growth, they also promote the health, appetite and general well-being of the fish. Oxygen also helps to reduce the effects of temperature-induced stress in fish.

Properly applied, oxygenation will reduce risk factors and increase profitability, but improper usage can easily backfire. Ultimately, oxygen is not a substitute for water – too much oxygen can be harmful. The proper oxygen dosing technology allows farmers to smoothly increase stock density, ensuring an optimal feed conversion ratio and the highest possible survival rates under optimum breeding conditions.

Therefore, both oxygen and oxygenation equipment, must be carefully and continuously optimized in close collaboration with the fish farmers in order to set up a commercial farming. Beside that all fish farmers must have a thorough understanding of the cause/effect relationship across all aspects of fish farming. Hereafter, Oxygen management and the way of applying Liquid oxygen in fish

Farms will be discussed. [43= Linde website]

2.1.6.Oxygen Management in Aquaculture:

A professional oxygen management will secure a successful fish farm. Supplying sufficient oxygen to sustain healthy fish and bacterial populations and to meet the biochemical oxygen demand (BOD) for fish waste and unconsumed food is critical. Maintain oxygen levels, near saturation or even at slightly super-saturation at all times. The amount of oxygen needed in a fish farm depends on a number of factors. Oxygen demand is directly correlated with the density of fish in the pool, feeding rates, water temperatures, flow rates, and the amount of waste product in fish pool. It is also a function of physical conditions such as water temperature and water volumes [44].

Increasing dissolved oxygen concentrations can be held in pool of fixed size through different methods. Below some of this method is mentioned:

Oxygen in depleted water can be increased by giving more chance for water and air to mix – fish pond owners in Asia often mix the surface water by beating with sticks and hands when they suspect oxygen depletion; mixing of water by electrical agitators and compressed air supply can also be effective. Water entering the pond can be made to cascade and splash down into the water for picking up more oxygen. Fertilizer application and blooms must be controlled so as to avoid the night time low of oxygen in ponds. Artificial lighting can cause increased photosynthesis and higher DO levels, but this is possible only in small often closed systems. Continuous injecting of fresh water as the inlet flew of the pools and increasing water flow rates (turnover times) must also be considered. Using aeration equipment, such as air blower or compressor with air stones, electric paddle wheel, and decreasing depth of water to accentuate wind action. Though, surface aerators may not be cost effective or efficient in evenly distributing oxygen throughout large commercial-scale systems. These are all the ways which can be applied to increase DO in fish pool [40].

By the way, the most commercial and practical way to keep the oxygen levels, near saturation or even at slightly super-saturation at all times in a fish pool is meet by using pure oxygen injection system. As it's shown in Table below the partial pressure of pure oxygen is five times higher than that of oxygen in air. As a result, oxygen dissolves more easily in water.

2.1.7.Pure Oxygen in Aquaculture:

Pure oxygen injection systems are increasingly being used in aquaculture. They are particularly useful in maintaining oxygen-saturated conditions in recirculating systems, raceways and cage culture with high densities of fish. Pure oxygen can be delivered and stored in a cryogenic tank as liquid oxygen or it can be produced on-site by an oxygen generator (Figure 2.1).

Bottled oxygen gas also is sometimes kept as an emergency backup system, this alternative usually is too expensive and bulky to be practical but it's better to be done.

Liquid oxygen technology is relatively simple, efficient, and cost-effective; especially if purchased in bulk quantities and if the site is located near a reliable supplier. A liquid oxygen system consists of a storage tank for the liquid gas, vaporizers to turn liquid oxygen to gas, a reactor to mix the produced oxygen gas with a part of inlet water, and supply lines to transfer the oversaturated water from the reactor to the fish pools [44].

Table 2.1.1 : Oxygen solubility (mg/l) in sea water and soft water in different temperatures. Source: Linde Gas Co. Aquaculture brochure. www.Linde-gas.com

	OXYGEN SOLUBILITY (mg/l)			
	SOFT WATER		SEA WATER	
WATER TEMPERATURE (°C)	AIR	PURE OCYGEN	AIR	PURE OCYGEN
11	11,0	52,4	8,9	42,4
12	10,8	51,4	8,7	41,4
13	10,5	50,0	8,5	40,5
14	10,3	49,0	8,3	39,5
15	10,1	48,1	8,2	39,0
16	9,8	46,6	8,0	38,1
17	9,6	45,7	7,9	37,6
18	9,4	44,7	7,7	36,7
19	9,3	44,3	7,6	36,2
20	9,1	43,3	7,4	35,2
21	8,9	42,4	7,3	34,7
22	8,7	41,4	7,1	33,8
23	8,6	40,9	7,0	33,3
24	8,4	40,0	6,9	32,8
25	8,3	39,5	6,8	32,4
26	8,1	38,6	6,7	31,9
27	8,0	38,1	6,6	31,4
28	7,8	37,1	6,4	30,5
29	7,7	36,7	6,3	30,0
30	7,5	35,7	6,2	29,5

If the fish famers raise the oxygen saturation in the farm from 90 to 100 percent, for example, fish production can increase by one third. But because the water in the enrichment systems is highly oversaturated with oxygen, it is enough to enrich about 10 to 30 percent of the inlet water with oxygen in order to achieve the desired 100 percent oxygen saturation. After oxygen enrichment in reactor, the water flows into the fish pool via a special instrument known as the Oxy-Stream. This instrument can be customized as far as dimension and capacity to fit the specific fish pool. Its specially shaped outlet nozzles create a circular flow in the tank. In this way, the oxygenated water is distributed quickly throughout the fishtank for a homogeneous mixture. Similar oxygenation systems exist for salt water [45].

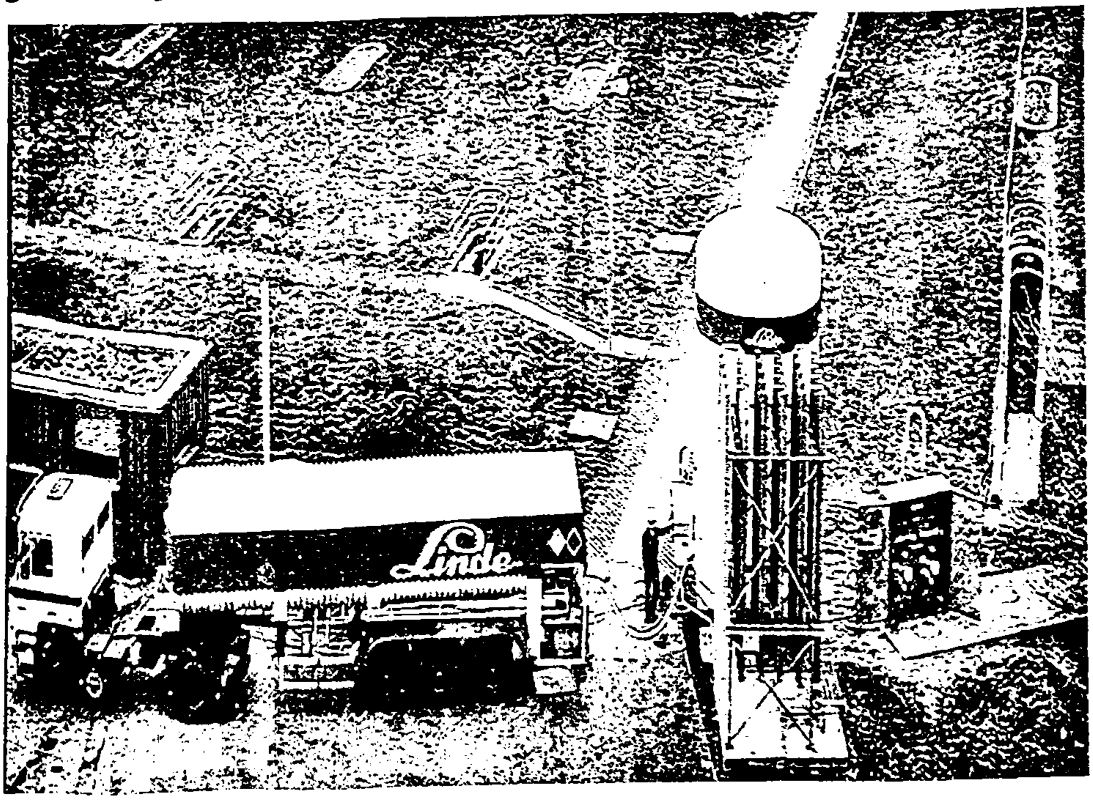

Figure 2.1: Delivering Liquid Oxygen to a Cryogenic Storage Tank.

The figure 2.2 shows the simplified diagram of pure oxygen injection system in a fish pool.

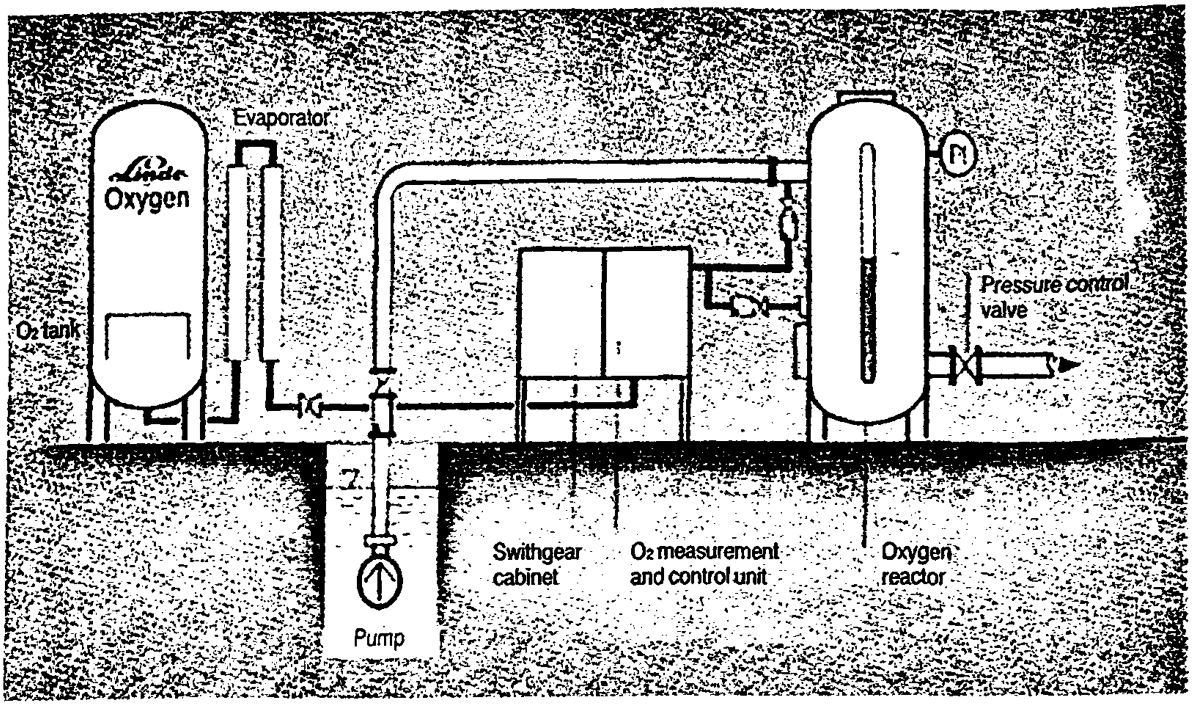

Figure 2.2: The pure oxygen injection system in fish pool. Source: Linde Gas Co. Aquaculture brochure. www.Linde-gas.com

It conveniently requires no external power supply and is therefore free of power failures and the consequent fish kills. As liquid oxygen temperature is -195ċ as soon as it enter the vaporizer from the Liquid storage tank it will transform to gas due to being in contact with atmosphere temperature. Therefore, the liquid oxygen transforms to oxygen gas without consuming any energy and that's money and energy consuming.

Figure 2.3 illustrate how pure oxygen injection system in installed and used in a small raceway fish farm in Iran.

Figure 2.3: A fish farm in Iran equipped with pure oxygen injection system.

Most fish farm owner rent or purchase a liquid oxygen storage tank and the related vaporizer of a size sufficient to provide two to four week supply of oxygen. The size of the tank corresponds with the fish production capacity of the system and the amount of oxygen which they need to provide enough saturated water depends on the density of fishes in the whole farm.

Oxygen generators (pressure swing adsorption systems, PSAs) are particularly advantageous at remote fish farms or the fish farms which have access difficulty specially in winter time liquid oxygen deliveries would be costly and expensive. Generators produce oxygen by using electric energy. They are expensive to purchase and operate, and they are subject to power failures. Selecting the proper size of the oxygen generator and carefully calculating the cost of electric power needed are important considerations.

2.1.8. Oxygen Diffusion in Water:

The technology that is used for oxygenation depends on such factors as whether salt- or freshwater fish are being raised, Significantly less energy is required to add oxygen to salt water than fresh. This is because the gas bubbles do not combine in salt water and thus remain small. As a result, the oxygen has plenty of time to dissolve in the water. In fresh water on the other hand, small gas bubbles rapidly combine into larger ones and rise quickly to the surface [45].

Effective diffusion of pure oxygen gas into water can best be accomplished using a U-tube oxygenation, counter-current flow injectors, Gas injection hose, or micro-bubble devices (tubes or fine wet stones). The purpose is to dissolve much of the oxygen injected so that it is available to the fish, rather than wasted by bubbling out of solution to the atmosphere. The function of the gas injection hose is shown in figure 2.4.

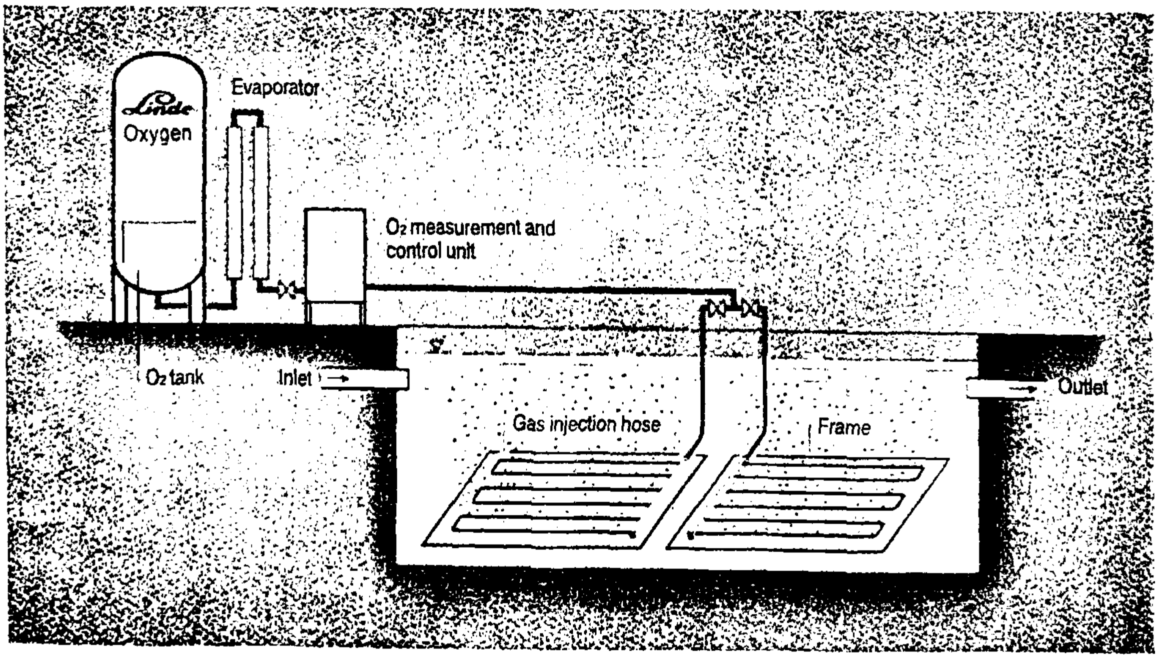

Figure 2.4: Gas injection hose system.

The U-tube in the Virginia Tech system consists of a shallow well (40 feet deep) into which tow concentric pipes (one outer 6 inch and an inner 3 inch diameter pipe) are suspended. The water, injected with oxygen at the top of the U-tube, flows down the inner pipe, up the outer pipe, and back into the fish culture tank saturated with oxygen. The U-tube increases oxygen transfer because of the longer contact time and greater atmospheric pressure (two atmospheres at the bottom of the U-tube) of the entrained oxygen gas bubbles and water. Although the U-tube aerator is not essential, oxygen enrichment (entrainment) is significantly increased by this cost-effective method [44].

Linde Gas Company as a leader in water oxygenation innovate a ceramic diffuser with commercial name SOLVOXRCD which is a high-performance, aluminium-housed oxygen dissolving equipment. Its high efficiency is a result of creating microscopic bubbles. Moreover, the flat plate design ensures uniform bubbles across the entire surface and minimizes bubble coalescence. It is therefore also suitable to dissolve oxygen into shallow fish pools down to water depths of less than 1 metre. The main field of application for the SOLVOXRCD is to provide additional oxygen to individual fish pool and raceways and for emergency oxygen supply (Figure 2.5) [46].

Generally all of this devices lead to produce a homogenize water saturated system in each individual fish pool. As a result fish will be distributed equally in all parts of the pool instead of gathering in inlet or outlet of each fish culture tank. The homogenized saturated water environment helps the fish farmers to distribute the food more easily in each pool.

The fish in such fish farms must also be specially protected from diseases, which would spread very quickly in the tanks. For that reason, ozone is often added to the water circulation to reduce pathogens in fish farms [45].

Ozone (O3) is a naturally occurring gas (upper atmosphere) that consists of three atoms of oxygen. It is a powerful oxidizing agent that can be used to break down compounds. Ozone must be used with caution since it is directly toxic to aquatic life and may form harmful bi products (hypochlorite, hypobromite).

Careful redox potential measurements and special injection equipment apparatus are needed to determine and control ozone applications [44].

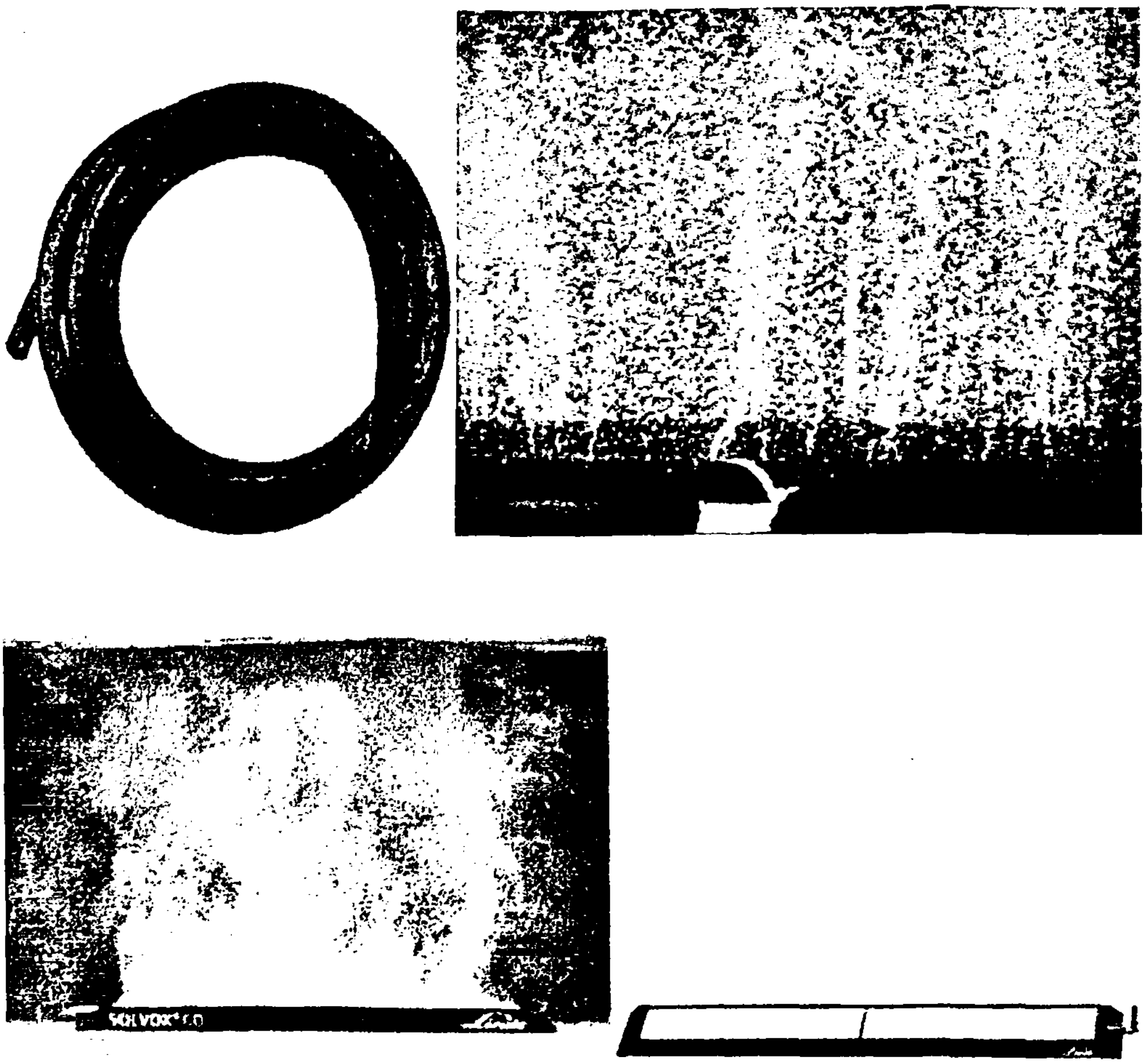

Figure 2.5: Gas Injection Hose Instrument.

Besides the oxygen content, temperature is another important factor that must be constantly monitored. If the fish pools become too warm, the water loses its capacity to dissolve gases. Tiny gas bubbles form, as in a glass of mineral water, but these bubbles are filled with nitrogen. Nitrogen dissolves easily in water and is released with heat. The nitrogen gas beads must be removed as quickly as possible, otherwise they can cause what is known as gas bubble disease.

This condition in fish can cause embolisms in the circulatory system or blindness. The problem can be prevented by the addition of pure oxygen.

Up to now, fish farming has been practiced mostly in so-called open systems, in which water flows into the breeding facility from open waters and back. But the trend is more toward closed systems. "In that case, 90 percent of the water remains in recirculation". Such systems – because they are almost completely closed off – are more environmentally friendly than open systems. In order to ensure safety in such closed facilities, carefully controlled water treatment is a requirement. Carbon dioxide and ammonia accumulate in the water of fish farms due to respiration and excretion. With nearly closed water circulation, these substances must be removed. This is done either by means of aeration (in the case of carbon dioxide) or through biological filtration (in the case of ammonia) [45].

In chapter 3 more details of dissolved oxygen effect on fishes will be investigate.

2.2. Food Technology:

Nowadays, good food has to be healthy, minimally processed and at attractively packaged – as consumers' expectations from foodstuffs are continuously rising. Consequently, demands on food producers and producers of packaging machines and materials are increasing, too. The consumer of today reacts sensitively when it comes to artificial additives. There is a strong trend towards being able to purchase and prepare fresh foodstuffs and ready-made dishes at any time. Food safety and easy access to all kinds of foodstuffs are very important. Therefore, it is becoming more and more difficult to meet consumers' great expectations. It is also becoming clear that the time factor is crucial. [48=Linde Mapax Catalogue]

The food industry is no exception with demand for all types of produce to be supplied to every inhabited place on the globe. Regardless of season and location, everything from exotic tropical fruits to the staple diet of bread, rice and potatoes are expected to be available all year round and in "just produced" condition at competitive, affordable prices. Convenience and quick preparation of meals is also a high priority for the fast paced 21st century lifestyle.

Attractively presented fresh or prepared foods and combination meals, in durable hygienic packages that offer useful shelf life under normal refrigeration, have become very popular.

Faced with these consumer preferences and rowing demand for an ever wider range of food products, retailers recognize the need for improvements in packaging technology. They need to address the spoilage problem and provide a huge diversity of new prepared foods.

Health concerns continue to drive the insistence on reducing salt, chemicals and preservatives which also places additional pressure on suppliers and manufacturers. They are expected to provide food products that look and taste like they were freshly made or just picked, despite possibly having travelled half way around the world by sea freight. Operating in a very competitive environment, from the independent retailer to global supermarket chains realize that minimizing waste by increasing shelf life, whilst ensuring the product is of prime appearance and taste, is essential to maximizing what is often very narrow profit margins. This can be difficult to achieve, but satisfied, loyal, returning customers are key, and both retail and commercial outlets are insisting more and more that their suppliers satisfy these demands with minimal financial impact [49].

Beside innovation and new mechanism in food Industry, industrial gases are increasingly giving an invisible helping hand to this sector.

The principal uses of food gases are as follows:

a. Additives

Oxygen, nitrogen and carbon dioxide as modified atmosphere packaging gases

Nitrogen and carbon dioxide as propellant gases for beverages

Nitrous oxide as a propellant gas for cream

Sulphur dioxide as a preservative for specified foods

b. Processing Aids

Liquid nitrogen and liquid carbon dioxide for freezing and chilling

Carbon dioxide for super-critical extraction

Hydrogen for hydrogenation of fats

c. Ingredients

Carbon dioxide for carbonated beverages,[50= [EIGA]

Hereafter, the role of these gases will be discussed in detail.

2.2.1.Food Industry:

The food industry is from farming and food production, packaging and distribution, to retail and catering. The food production of today has to face ever-growing expectations of customers, increased competition and rising safety and environmental standards.[47= linde cryoline catalogue]
The better the packing, the more benefit regarding to added value.

2.2.2. Food Grade Gases :

Food gases are defined as gases, in liquid, gaseous or solid form that are supplied to the food industry and used as additives, processing aids or ingredients in contact with food. These include gases for modified atmosphere packaging, liquid nitrogen for freezing and carbon dioxide for beverage carbonation. They may be delivered as bulk liquid gases, compressed cylinder gases, generated on-site or, in the case of carbon dioxide, as solid dry ice [50].
Food-grade gases must conform with 'food grade' regulations, e.g. the EC directive 96/77/EC on food additives within EU countries and the FDA guidelines in the U.S [48].

The main Industrial gases which is using in food industry are including Food grade carbon dioxide (CO_2), nitrogen (N_2), and oxygen (O_2) along with other gases authorized for foodstuffs as individual gases in cylinders under high pressure as well as liquids in cryogenic storage tanks for subsequent mixing at the packaging machine and premixed.

The cryogenic characteristics of liquid nitrogen and carbon dioxide are used for cooling and freezing, tumbling, cutting, mixing and cooling during transport. Cryogenic freezing of food with liquid nitrogen (LIN) and carbon dioxide (LIC) relies on those gases' extreme cold temperatures as they come into contact with food. In the case of liquid carbon dioxide, snow forms when the liquid expands. The snow then vaporizes on the product.

Nitrogen and oxygen are separated from atmospheric air. Carbon dioxide is taken from natural wells or captured as a byproduct of fermentation processes (wine, beer) or ammonia production. For example, sometimes it may be more effective and practical to produce nitrogen on-site using pressure swing absorption (PSA) or a permeable membrane plant.

Microorganism growth can also be inhibited to a certain extent with the help of other gases authorized for foodstuffs, such as nitrous oxide, argon or hydrogen. Each of the gases has its own unique properties that affect its interaction with the foodstuffs.

The food grade gas supply option that may be best depends on the type of foodstuff, the production volume, the packaging line and also whether the gas is to be used anywhere else in production. It may be preferable to have premixed gases supplied if production is relatively limited or if a new production facility is being started up. When production rates increase and various products are to be packaged, it may be more suitable and more economical to switch over to mixing gases on site. Then a mixer is used and the gases are supplied from cylinders, tanks or PSA/membrane systems. Each application must be evaluated separately before decisions can be made regarding the supply options and gas Mixtures [48].

As follows the application of food grade gases and their effect in food industry will be investigated.

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2.2.3 : Modified Atmosphere Packaging (MAP) :

Food is a biological, sensitive substance. Original freshness and shelf-life are affected by the inherent properties of the product just as much as by external factors. It is primarily microbial and chemical and biochemical deterioration that destroys food [48].

Many foodstuffs start to deteriorate the moment they are harvested or manufactured and prepared for packaging. The cause is attack from a multitude of spoilage mechanisms such as bacteria, yeast and mold spores, either airborne or naturally occurring within the product. Moisture loss or gain, depending on the food can also be undesirable [49].

The presence of microorganisms can be traced back to the raw materials, the ingredients and the environment. Microorganisms are found everywhere in our surroundings, e.g. on our skin, on tools and in the air. For this reason, good hygienic conditions must be maintained throughout the processing chain [48].

If these spoilage mechanisms can be excluded, prevented or their progress retarded in some way, then the natural process of food decay can be delayed, allowing more time from production to consumer without affecting quality. This benefits everyone in the supply chain from grower, manufacturer and Industrial gas companies through to logistics, retailer and ultimately, the consumer. Over the past three decades, a safe, tried, tested and proven method of combating food spoilage mechanisms without the use of (or at least a substantial reduction in) undesirable preservatives, is the use of Modified Atmosphere Packaging. Sometimes referred to as "MAP" or "Gas Flushing". MAP is quite literally a process whereby produce is packed or stored in a "modified" form of the Earth's naturally occurring air. The normal ratio of gases is modified to significantly reduce or prevent the effects of spoilage mechanisms. However it must be stressed that MAP is not a solution on its own. The shelf life of food produce is influenced by a number of factors including:

- Storage temperature
- Quality of raw ingredients
- Product formulation
- Processing method
- Hygiene standards
- Packaging Material,

If any of these are lacking or substandard, the benefits of MAP can be reduced or even totally compromised [49].

The main gases used in Modified Atmosphere Packaging (MAP) applications are carbon dioxide, nitrogen and oxygen. These gases are used either alone or as a mixture of mentioned gases. The gas properties and the interaction of gases with the food ingredients, e.g. solubility in the foodstuff, should be taken into account when choosing the gas or gas composition. The gas atmosphere must be chosen with due consideration of the particular food stuff and its properties. For low-fat products with high moisture content, it is especially the growth of microorganisms that has to be inhibited. On the other hand, should the product have high fat content and low water activity, oxidation protection is most important.

Carbon dioxide is the most important gas in the field of MAP technology. Most microorganisms such as mold and the most common aerobic bacteria are strongly affected by carbon dioxide. The growth of anaerobic microorganisms, on the other hand, is less affected by this gas atmosphere. Carbon dioxide inhibits microbial activity by effectively dissolving into the food's liquid and fat phase, thereby reducing its pH-value, and by penetrating biological membranes, causing changes in permeability and function [48].

Nitrogen is the most widely used gas. With the exception of raw red meat, raw offal, dark poultry cuts and hard cheese, nitrogen is used in some way for every other food that can benefit from MAP [49].

Nitrogen is an inert gas. It is primarily used to replace oxygen in packaging and thereby prevents oxidation. Owing to its low solubility in water, nitrogen also helps to prevent package collapse by maintaining internal volume.

Oxygen mainly is bad, sometimes in MAP technology. Good For most foodstuffs, the package should contain as little oxygen as possible to retard the growth of aerobic microorganisms and reduce the degree of oxidation. However, there are exceptions. Oxygen helps to preserve the oxygenated form of myoglobin, which gives meat its red color. Oxygen is required for food and vegetable respiration.

Basically, microorganisms can be divided into two categories: aerobic and anaerobic. Aerobic organisms require the presence of oxygen (O2) to survive and multiply. Anaerobic organisms, on the other hand, grow in the absence of oxygen. Aerobic microorganisms include Pseudomonas, Acinetobacter and Moraxella which spoil food by decomposing and producing substances that give a bad taste and odor. Anaerobic microorganisms include Clostridium and Lactobacillus. When foodstuffs are handled incorrectly, Clostridium can generate a toxin. Lactobacillus, on the other hand, is a harmless bacterium that turns the food sour by producing lactic acid [48].

These gases or mixture of two or all three will be required in MAP technology is required to control four main types of micro-organisms as follows:

Aerobic Microbes: These need air or oxygen to respire and grow. Displacing the air in the packaging or storage process with nitrogen gas will cause a reduction in the oxygen level to a point where the bacteria are suppressed and the desired extended produce life is achieved. Some Bacillus, for example.

Anaerobic Microbes: These live without air or oxygen. Some species may even be destroyed or inhibited by very low levels of oxygen. Clostridium, for example.

Microaerophilic Microbes: These need low levels of oxygen to provide an optimum environment for growth. Some also require elevated levels of carbon dioxide, Campylobacter, for example.

Facultative Anaerobic Microbes: These can live and grow with or without air or oxygen. Salmonella species, for example [49].

MAP is used to increase the shelf life of food, comparison of shelf-life for products packed in air and with MAP respectively shown in table 2.2.

2.2.4. Other Application of Nitrogen Gas in Food Industry:

The other Nitrigen application in food industry is listed below.

2.2.4.a. Controlled Atmosphere (CA) storage:

Large gas tight temperature and oxygen controlled bulk stores are typically used for fruit, vegetables and salads. Purging with nitrogen gas removes oxygen and CO2 which will slow product deterioration from weeks to many months.

In addition to land based CA stores, marine units provide the same level of benefit on-board specially modified cargo holds within ships, allowing the most economical transportation of perishable foods from all areas of the world.

2.2.4.b. Sprigging:

Nitrogen gas is passed through liquids such as cooking oils to help reduce dissolved oxygen. Diffusers are used within the oil storage vessels to ensure small bubbles of nitrogen gas are produced to achieve the best results.

2.2.4.c. Pressure transfer:

Nitrogen gas is used as an inert, non-oxidizing motive force to convey powders and liquids where it is undesirable or not possible to employ traditional pumping methods. Using nitrogen gas gives the additional benefit of fire and explosion suppression, often associated with powders, dust and flammable liquids.

Table 2.2.1: comparison of shelf-life for products packed in air and with MAP.

Typical shelf-life with MAP	Typical shelf-life in air	Food
5-8 days	2-4 days	Raw Red meat
16 – 21 days	4-7 days	Raw Light Poultry
7-14 days	3 – 5 days	Raw Dark Poultry
2-5 weeks	2-4 days	Sausage
2-5 weeks	2-4 days	Sliced cooked meat
5-9 days	2-3 days	Raw Fish
3-4 weeks	2-4 days	Cooked Fish
4-10 weeks	2-3 weeks	Hard Cheese
1-3 weeks	4 – 14 days	Soft cheese
up to one year	Several weeks	Cakes
2 weeks	Some days	Bread
20 days	5days	Pre-baked bread
5 – 10 days	2- 5 days	Fresh cut salad mix
3 – 4 weeks	1 – 2 weeks	Fresh pasta
2 – 4 weeks	7 – 10 days	Pizza
2 – 3 weeks	3- 5 days	Pies
7 – 10 days	2-5 days	Sandwiches
7 – 20 days	2-5days	Ready meals
1 -2 years	4-8 months	Dried foods

2.2.4.d. Silo and bulk storage blanketing:

Providing an inert nitrogen gas "blanket" at minimal overpressure, above food produce contained in bulk storage silos or vessels will help prevent oxidization and contamination from possible external atmospheric sources.

2.2.4.e. Insect and larvae reduction:

Storage of produce such as cereals and grains can be purged and blanketed with nitrogen gas to eradicate insects or the development of their larvae. While the vast majority of these pests are totally harmless to consumers, minimization of their presence is often desirable.

2.2.4.f. Nitrogen injection:

Nitrogen gas is often used to create micro bubbles in products such as cream and certain desserts to increase bulk and improve texture. Nitrogen is used in preference to air as it is less likely to be absorbed into the product, therefore maintaining the bulk for longer, and as it is inert, it is less likely to oxidize the product and affect the taste.

2.2.4.g. Aerosol propellant:

Nitrogen gas is used as an inert propellant in aerosol dispensed products such as "squirty" creams, oils and cheeses.

2.2.5. Food Freezing and Cooling:

In modern food processing, cryogenic freezing and cooling has become a well-established technology to increase product quality. Modern freezer designs use the latest control programs to increase efficiency while meeting stringent hygiene standards. Cryogenic freezing of food with liquid nitrogen (LIN) and carbon dioxide (LIC) is a well-established practice that relies on those gases' extreme cold temperatures (–196°C or –320°F, in the case of liquid nitrogen) as they come into contact with food. In the case of liquid carbon dioxide, snow forms when the liquid expands. The snow then vaporizes on the product [43].

Liquid Nitrogen used in many applications across a broad range of industries, though perhaps the most profound effect of nitrogen however has occurred in the last 100 years is for freezing and chilling food.

Liquid Nitrogen is best utilized in processes where extremely low temperatures or rapid rates of cooling are required. In many cases the use of Liquid Nitrogen freezing , or Cryogenic freezing as it is known, is a preferred alternative to the use of Mechanical Freezing devices such as traditional refrigeration units. Cryogenic freezing has traditionally been attractive due to its low capital costs as units tended to be far smaller and cheaper to produce. Gas producer companies have been able to gain large refrigeration capacity at little or even zero capital cost, simply by renting Cryogenic storage tanks from gas companies and buying Liquid Nitrogen to suit demand.

Major players in the food industry have used Liquid Nitrogen for freezing meats, fish, poultry, dairy and bakery products and many others such as pasta, prepared meals (microwave meals), fruit and vegetables.

In some cases the very rapid freezing with Liquid Nitrogen has very marketable benefits. Such as the prevention of crystal formation on food stuffs which causes cell damage to the product. Liquid Nitrogen prevents oxygen from reaching the food's surface this denies bacteria the oxygen they need to grow and multiply. Where mechanical freezers can cause dehydration of products cryogenic freezers can avoid any dehydration, which in turn provides producers with a better yield.

Apart from the frozen foods sector there is a vast and rapidly expanding market for Liquid Nitrogen in the 'chilling' of foods. For fresh fruit and many convenience foods that do not withstand or even require freezing food chilling equipment is required. For fresh fruit in particular on site chilling solutions are required to prevent field heat deterioration. Several ground and diced food products are also cryogenically chilled in blenders using Liquid Nitrogen [51].

Cryogenic freezing and cooling of foodstuffs using liquid nitrogen and carbon dioxide is has been enhancing food processing for many years (figure 2.6). Benefits of fast freezing by the help of these two liquid gases are listed below:

- Preserving food flavor, texture and odor.
- Reducing the risk of food spoilage and improving food safety.
- Higher quality of the frozen product compared with slower methods.
- Reduced drip loss on defrosting.
- Less dehydration resulting in a higher yield.
- Lower space requirements.

Figure 2.6: Frozen Fruit.

For food transportation with help of Liquid nitrogen (LIN) there's a powerful cooling technology that indirectly uses the cryogenic effect of to provide rapid and evenly distributed temperature pull-down. It also has the ability to maintain accurate product temperature throughout the truck compartment despite multiple delivery stops, reducing the risk of food spoilage and improving food safety [43].

CHAPTER 3. APPLYING INDUSTRIAL GASES:

In the following chapter the application of Industrial gases in food industry and Oxygen in Aquaculture, our practical experiments and the related procedure are described.

3.1. Fish Breathing and Oxygen Consumption:

The effect of Oxygen in fish breathing and the reaction of fishes to hypoxia and hyperoxia show the important role of the right level of oxygen in fish growth, the food consumption and the food conversion ratio.

3.1.1. Absorption of Dissolved Oxygen by Fish Gill:

For most fish species gills work by a unidirectional flow of water over the epithelial surface of the gill, where the transfer of gases occurs (O_2 in, CO_2 out). The reason for this unidirectional flow of water is the energetic nature of the system. The energy that would be required to move water into and out of a respiratory organ would be much more than that used to move air because water holds low oxygen due to its low solubility [53].

The blood flowing just under the epithelial gill tissue usually moves in a counter current flow to that of the water moving over it. This allows for most of the O_2 to be taken in by the blood because the diffusion gradient is kept high by the blood picking up oxygen as it moves along, but always coming into contact with water that has a higher O_2 content. The blood receiving the O_2 continues to pick up O_2 as it moves along because fresh water is being washed over the epithelial lining of the gills [54].

By doing so, the fish ventilate the gills while also taking in oxygen and releasing carbon dioxide [53].

However there are two ways fish ventilate their gills: buccal/opercula pumping (active ventilation) and ram ventilation (passive ventilation). In buccal/opercula ventilation the fish pull in water through the mouth (buccal chamber) and push it over the gills and out of the opercula chamber (where the gills are housed). At this time the pressure in the buccal chamber is kept higher than the pressure in the opercula chamber so as to allow the fresh water to be constantly flushed over the gills. In ram ventilation, a fish swims with its mouth open, allowing water to wash over the gills. This method of ventilation is common to fast moving fish, and it enables tuna to keep enough oxygen going to the gill surface while swimming at high speed [55].

During this time the oxygen is absorbed into the blood while carbon dioxide diffuses out of the blood to the water. The pathway taken by carbon dioxide and explain that, in the blood, CO_2 is transported in the form of bicarbonate. The bicarbonate moves from the blood by passing through the erythrocytes in which O_2 binds to Haemoglobin (Hb) at the respiratory surface, causing hydrogen ions (H^+) to be released. The increase in H^+ ions combines with HCO_3^- to form CO_2 and OH^-. Thus, more CO_2 is formed and can leave the blood across the respiratory surface. Excess H^+ binds to OH^-, forming water and allowing the pH to increase enough to promote the binding of oxygen to Hb. The release of O_2 from Hb in the tissues makes the Hb available to bind to H^+, promoting the conversion of CO_2 to HCO_3^-, which helps draw CO_2 from the tissues. Therefore, CO_2 that is being transported into and out of the red blood cells minimizes changes in pH in other parts of the body because of proton binding and proton release from hemoglobin, as it is deoxygenated and oxygenated, respectively. However, carbon dioxide is rarely a problem in when dissolved oxygen concentrations are well above saturation levels. Due to these processes, oxygen level should be kept at or a little bit higher during the entire culture period [53].

3.1.2. Effects of oxygen level on oxygen uptaking by fish:

It is commonly thought that if there is not enough oxygen in the water, then the fish will be seen gasping at the surface but this is a last resort means to breathe. The first indication there may be a dissolved oxygen problem in the water is when the fish become unusually lethargic and stop feeding. As oxygen levels decrease, the fish do not have enough energy to swim and feeding utilizes yet more oxygen. Often it is recognized the fish have a problem at this stage but frequently some form of medication is added to the water and this can actually cause the oxygen level to drop even lower, leading to a number of mortalities. This can lead to the mistaken conclusion that the fish were suffering from some form of disease. In terms of managing any aquatic system, it is always advisable to increase the aeration when any fish start to behave abnormally, before adding any form of medication to the water. Increasing the aeration will certainly make the environment more comfortable for the fish, even if the dissolved oxygen level was already satisfactory. With respect to improving the aeration before adding medication, this will allow for any depletion of the oxygen level caused through a chemical reaction with the medication . As it said before the recommended minimum dissolved oxygen requirements are as follows:

Cold water fish - 6 mg per litre (70% saturation) ,Tropical freshwater fish- 5 mg per litre (80% saturation) and Tropical marine fish- 5 mg per litre (75% saturation).

It is worth bearing in mind that these values are minimum requirements for healthy growth, tissue repair and reproduction [56].

Most fish species will tolerate a drop below these minimum values for a short period of time, probably the cold water species are likely to tolerate a lower level than tropical fish. However, the period of time during which the oxygen level drops below the required minimum level, will cause the fish to become stressed. It is this stress which causes fish death. It may take the fish several days to recover from short term oxygen depletion but where the levels are persistently low, an assortment of stress related diseases such as fin rot and white spot may occur.

3.1.3: Fish reaction to hypoxia:

Hypoxia or oxygen depletion is a phenomenon that occurs in aquatic environments as dissolved oxygen (DO) becomes reduced in concentration to a point detrimental to aquatic organisms living in the system. An aquatic system lacking dissolved oxygen (0% saturation) is termed anaerobic. Reducing or anoxic is a system with a low DO concentration in the range between 1 and 30%. DO saturation is called hypoxic. Most fish cannot live below 30% DO saturation. A "healthy" aquatic environment should seldom experience DO of less than 80%. In response to a low concentration of dissolved oxygen in the water, the fish can respond in two ways: the blood flow can be increased by opening up further secondary lamellae to increase the effective respiratory area (it may be difficult to increase significantly the blood flow rate through the capillaries themselves), and the concentration of red blood corpuscles can be increased to raise the oxygen carrying capacity of the blood per unit volume. The latter can be achieved by reducing the blood plasma volume (e.g. by increasing the urine flow rate) in the short term, and by releasing extra blood corpuscles from the spleen in the longer term [56].

At the same time, the ventilation rate is increased to bring more water into contact with the gills within a unit of time. There are, however, limits to the increased flow attainable; the space between the secondary lamellae is narrow (in trout it is about 20 μm) and water will tend to be forced past the tips of the primary lamellae when the respiratory water flow is high, thus by-passing the respiratory surfaces. These reactions are quite adequate to compensate for the normal fluctuations of energy demands of the fish and of dissolved oxygen concentrations in the water. One of the consequences, however, of an increased ventilation rate is that there will be an increase in the amount of toxic substances in the water reaching the gill surface where they can be absorbed [55].

However, oxygen deficiency causes asphyxiation and fish will die, depending on the oxygen requirements of the species and to a lesser extent on their rate of adaptation. Fish exposed to oxygen deficient water do not take food, collect near the water surface, gasp for air (cyprinids), gather at the inflow to ponds where the oxygen levels are higher, become torpid, fail to react to irritation, lose their ability to escape capture and ultimately die. The major pathological-anatomic changes include a very pale skin color, congestion of the cyanotic blood in the gills, adherence of the gill lamellae, and small haemorrhages in the front of the ocular cavity and in the skin of the gill covers. In the majority of predatory fish the mouth gapes spasmodically and the operculum over the gills remains loosely open [56].

More than that, fish reduce food intake, leading to a reduction in growth. Reproduction is inhibited, and both fertilization success and larval survival are compromised. Energy utilization is decreased, associated with a shift from aerobic to anaerobic metabolism. To reduce energy expenditure under this situation, fish move to water at lower temperature, and reduce activity, reproduction, feeding, and protein synthesis. Transcription is reduced, mediated by increased levels of hypoxia-inducing factor 1 (HIF-1), which also up-regulates genes involved in erythropoiesis, capillary growth and glucose transport. All these responses are directed at maintaining cellular oxygen homeostasis and reducing energy expenditure, thereby augmenting survival of the animal during hypoxia. In general, the actions of toxicants are exacerbated during hypoxia, through a variety of mechanisms. Some species are much more tolerant of hypoxia than others, leading to differential survival during extended periods of hypoxia [57].

To avoid this, aquaculture systems have to be supplied with enough oxygen saturation. However, too much oxygen is also harmful to fish.

3.1.4: Fish reaction to hyperoxia:

Hyperoxia is the state of water when it holds a very high amount of oxygen. At this state, water is described as having a dissolved oxygen saturation of greater than 100%. This percent can be 140-300%. At this water condition, oxygen molecules will begin to move around within the water column looking for a little elbowroom. If there is non-available, it will return to the atmosphere or attach to the organisms around [34].

If fish are exposed (at a lower atmospheric pressure) to such water, their blood equilibrates with the excess pressure in the water. Bubbles form in the blood and these can block the capillaries; in sub-acute cases the dorsal and caudal fin can be affected, and bubbles may be visible between the fin rays. The epidermal tissue distal to the occlusions then becomes necrotic and cases are known where the dorsal fins of trout have become completely eroded. In severe cases, death occurs rapidly as a result of blockage of the major arteries, and large bubbles are clearly seen between the rays of all the fins. The remedy is either to remove the fish to normally equilibrated water or to provide vigorous aeration to strip out the excess gas [56].

In some species such as salmons and fast swimming fishes, the swim bladder acts like an oxygen store, to be used during the hypoxia. When the gads level in the blood is high gases will diffuse from the blood to the bladder. When the water is supersaturated (hyperoxia) the bladder becomes over-inflated and this leads to buoyancy problems especially in small fishes [53].

3.1.5. Effects of oxygen level on growth and food conversion ratios of fish:

Successful fish production depends on good oxygen management. Oxygen is essential to the survival (respiration) of fish, to sustain healthy fish and bacteria which decompose the waste produced by the fish, and to meet the biological oxygen demand (BOD) within culture system. Dissolved oxygen levels can affect fish respiration, as well as ammonia and nitrite toxicity. When the oxygen level is maintained near saturation or even at slightly super saturation at all times it will increase growth rates, reduce the food conversion ratio and increase overall fish production.

3.1.5.a. Effect of oxygen on fish growth:

Oxygen is important in respiration and metabolism processes in any animal. In fish, the metabolic rate is highly affected by the concentration of oxygen in the rearing environment. As the dissolved oxygen concentration decreases, respiration and feeding activities also decrease. As a result, the growth rate is reduced and the possibility of a disease attack is increased. However, fish is not able to assimilate the food consumed when DO is low [58]. Overall health and physiological conditions are best if the dissolved oxygen is kept closer to saturation. When the levels are lower than those mentioned above, the growth of the fish can be highly affected by an increase in stress, tissue hypoxia, and a decrease in swimming activities and reduction in immunity to diseases. However, there is a need to maintain the level of dissolved oxygen at the saturation level which will not affect its physiological or metabolic activities, so as to have high production in any culture system [59]. More than that, one has to keep in mind that the oxygen level requirement depends on the species, but also on fish size and activity of the fish. Oxygen requirements per unit weight of fish significantly decline with increasing individual weight. In carp this reduction may be expressed by the following ratios: yearling = 1, two-year-old carp = 0.5–0.7, marketable carp = 0.3–0.4. Significant differences in oxygen demand are also found for different species. Using a coefficient of 1 to express the oxygen requirement of common carp, the comparative values for some other species are as follows: trout 2.83, pike perch 1.76, roach 1.51, sturgeon 1.50, perch 1.46, bream 1.41, pike 1.10, eel 0.83, and tench 0.83 [58].

Several studies have investigated the relationship between oxygen saturation and fish food intake. found that feeding patterns of channel catfish varied with temperature and oxygen availability. When the oxygen content drops below 59% fish starts to lose its appetite [60].

Rainbow trout (*Oncorhynchus mykiss*) reduced its appetite when oxygen saturation fell below approximately 60% [54].

Similar results have been obtained from European sea bass (*Dicentrarchus labrax, L*) [61], blue tilapia (*Oreochromis aureus*)[62], channel catfish (*Ictalurus punctatus*) [63], juvenile turbot [64] and common carp (*Cyprinus carpio. L*), showed reduced growth when exposed to low oxygen levels.

3.1.5.b.Effect of oxygen on the food conversion ratio in fish:

Fishes will regulate their metabolic rate over a range of dissolved oxygen concentrations; however, at some point, a further reduction in oxygen tension will produce a shift from a metabolic rate that is independent of oxygen concentration to one that is dependent on oxygen level. The point is referred to as the critical oxygen tension [65].

Decreased oxygen availability is also considered a major factor in determining food intake. Low dissolved oxygen is a type of stress frequently found in fish farms characterized by high fish densities and polluted fresh or marine waters. The food conversion ratio (FCR) is the amount of fish food consumed to generate a given weight gain. It is the ratio between the weights gained in a given period to the total feed intake by the fish in the same period. It is the inverse of the feed intake. The food conversion ratio is improved (lowered) at higher growth rates. The average FCR from several recent studies of fish growth is 0.97. It is well known that tilapia can tolerate hypoxic and even anoxic conditions for short periods and are thus better suited than other species to hypereutectic conditions that may exist in static water aquaculture systems [66].

3.2: Oxygen Experiment In a Trout Farm In Iran:

In order to practically observe the effect of dissolved oxygen on fishes we stared our experiment in a fish farm in Iran. Needless to say as this experiment has been done for the first time in Iran convincing a fish farm owner to install our oxygen diffusion system in his farm was a hard job!!!

3.2.1: Practical applying of dissolved oxygen in a trout farm in Iran:

Produced fish in this farm were Rainbow Trout (*Oncorhynchus mykiss*). This experiment had been accomplished by the contribution of Linde Gas Company and a trout farm in south west of Iran. All the necessary facilities including Liquid Oxygen Storage tank, vaporizer, the reactor, piping and the related installation have been operated by Linde Gas Co.

The Rainbow trout farm is located in Fars Province, 45 km far farm Shiraz. In this farm the rainbow trout is rearing in 29 race ways. In this experiment the inlet water of 7 race ways has been enriched by liquid oxygen (Group A), 7 Raceways has been filled with the without any oxygen treatment (Group B). The rest of pools were empty due to shortage of amount of inlet water.

During the 6 Months of this experiment, growth rate, heath condition and feed cost of fishes has been investigated. Also, it has been seen a huge difference in the capability of holding the amount of market size fishes (kg) in each m3 of enriched water by oxygen (Group A) in compare with Group B. The result also shows the extremely positive effect of applying liquid oxygen on growth and health condition of fishes in Group A.

3.2.2: Trout Farm specifications:

The trout farm is located in Fras Province, south west of Iran. It is consist of 29 pools with 3m width, 30 m length and 70 to 120 cm depth. Only 50cm of the depth was filled by water due to shortage of inlet water resources. Therefore, the available rearing area was 1,305 m3, theoretically this farm has the capacity of producing at least 13,050 kg trout fishes in each rearing period (6months). But the fact is that the farm owner could produce only 7 tons Rainbow trout per year. Although, the farm is located in a very good geographical location but the amount of dissolved oxygen in inlet water was only 5.5ppm which never can meet the needs of Rainbow Trout. So, practically this farm was unprofitable.

The main source of water in this farm was a spring 300m far from the pools. As the spring location is in a higher level than pools, the inlet water running to the pools by the of gravity of earth. The problem was that in the whole way from the spring to the pools the running water was covered by cement blocks, so the water didn't have any chance to be in contact with the air in order to exchange the gases, especially to absorb Oxygen from atmosphere.

Despite the dissolved oxygen shortage in the inlet water, the other characteristic of farm were not ideal but good enough for rearing of rainbow trout. Water temperature was between 12-14 Ċ, the water flow was 150lit/min, and the PH was 7. The whole amount of inlet water was 100 lit. It took 2 hours to exchange the whole water of each pool by fresh water which was not a very appropriate time, as it caused the increasing of NH3 in the pools. And as we all know NH3 is the most toxic element in rainbow trout farms.

Liquid oxygen in fish farms has been applied for the first time by our group in Iran. It was very difficult to convince the Iranian fish producers to provide us their facilities in order to investigate our study. For most of the fish producers their farm is the only source of income. As they afraid to lose their fishes which is all their wealth, it was rational if they afraid to participate in this experiment!!!

The obvious blind point of this Rainbow trout farm which we finally choose was the shortage of dissolved oxygen in the water. So, this farm was the best pilot for us to observe how liquid oxygen can be essential to change an unprofitable farm to a productive and beneficial one with the same facilities. On the other hand, the fish farmer could run his farm and achieve some profit out of it. Actually, this experiment was a win to win game!!!

Therefore, we started to enrich the inlet water of 7 raceways by Liquid Oxygen (Group A) and then we investigated the differences between harvested fishes from these raceways and the ones from those raceways without oxygen treatment (Group B).

3.2.3: Material and Methods:

Based on the fundamental information of the farm, also the background and history of the farm and the rearing duration of fishes till they come to market size (350gr), the amount of monthly needed Liquid oxygen has been investigated. Therefore, the proper size of Liquid Oxygen Storage tank for the adequate amount of oxygen for each one month consumption, the related vaporizer and the reactor has been selected. In this trout farm, approximately 3 tons of liquid oxygen could meet the needs of fishes. So, the smallest size of Liquid Storage tank and the related vaporizer was the best choice. The foundation of Liquid storage tank has been designed and prepared. Finally Liquid oxygen storage tank has been installed as it's shown in Figure 3.1.

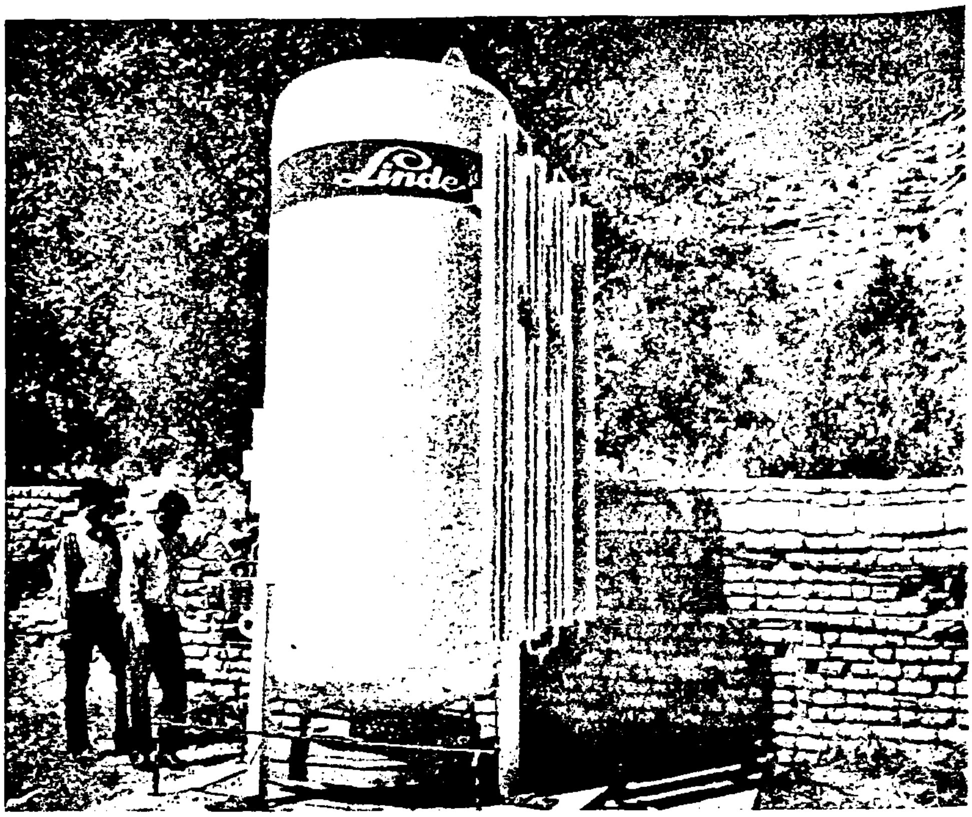

Figure 3.1: Liquid Storage Tank in Rainbow trout farm in Iran.

A semitrailer monthly arrived to the fish farm to refill the liquid stora
So, the Storage tank was full of Liquid oxygen during the whole experir

Due to different pressure level between Liquid Storage tank and the atmospheric pressure, the Liquid oxygen went out from the storage tank Storage tank piped to vaporizer, liquid oxygen directly entered the va Vaporizer consists of different quantity of wings based on the Liquid consumption. The duty of vaporizer is to transformed the Liquid oxyge Oxygen by passing the liquid oxygen through its wings.

Liquid oxygen (-182Ċ) passed through the vaporizer wings an transformed to oxygen gas with exactly the same temperature of atmosphere. In this process without applying any expensive cost electricity, gas oxygen is obtained.

As cupper doesn't have any ccorrosive reaction in contact with gas oxygen for transferring this gas in the whole experiment the cupper pipeline was utilized. After vaporizer through a cupper piping gas oxygen transferred to thecontrol panel (Figure 3.2).

Inside the control panel the flow rate of gas oxygen inside the pipeline and the outlet pressure of gas oxygen from control panle to reactor has been montitored respectively by the use of installed flowmeter and manometer. It was possible to change the flow rate and outlet pressure of gas oxygen based on the fish needs.After checking all the neccesary parameters inside the control panel the gas oxygen entered the reactor.The reactor played a very important role in the proccess of enrichment of inlet water.

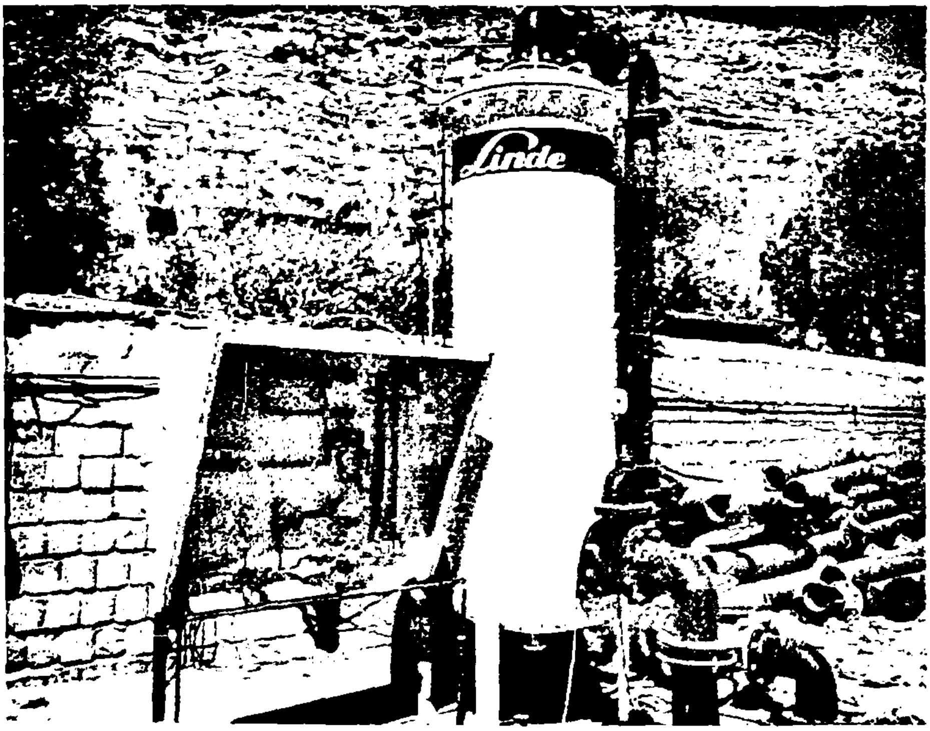

Figure 3.2: The contorl panel and the the Reactor in Rainbow Trout Fish farm in Iran.

As it's shown in Figure 3.3, gas oxygen enterred to the top of the reactor, 1/ of the whole amonut of farm inlet watre (30lit) also pupmed to the top of the reactor. Therefore, the top of reactor was the first point which both g oxygen and inlet water combined together. Approximately 30cm from the t of the reactor a mesh disk has been fixed. This disk was composed of ve small holes. The mixture of water and gas oxygen passed through these sm holes and combined very well together. The turbulenec that happened inside t reactor caused the water to be in a very close contact with oxygen, therefore t water inside the reactor became supersaturated. The concentration of oxygen the water inside the reactor was 40ppm.

The supersaturated water (1/3 of total amount of farm water source) transfen form the reactor to the 7 raceways in Group A. This supersaturated water l been directed from the reactor through a main PVC pipline. Figure 3.4.

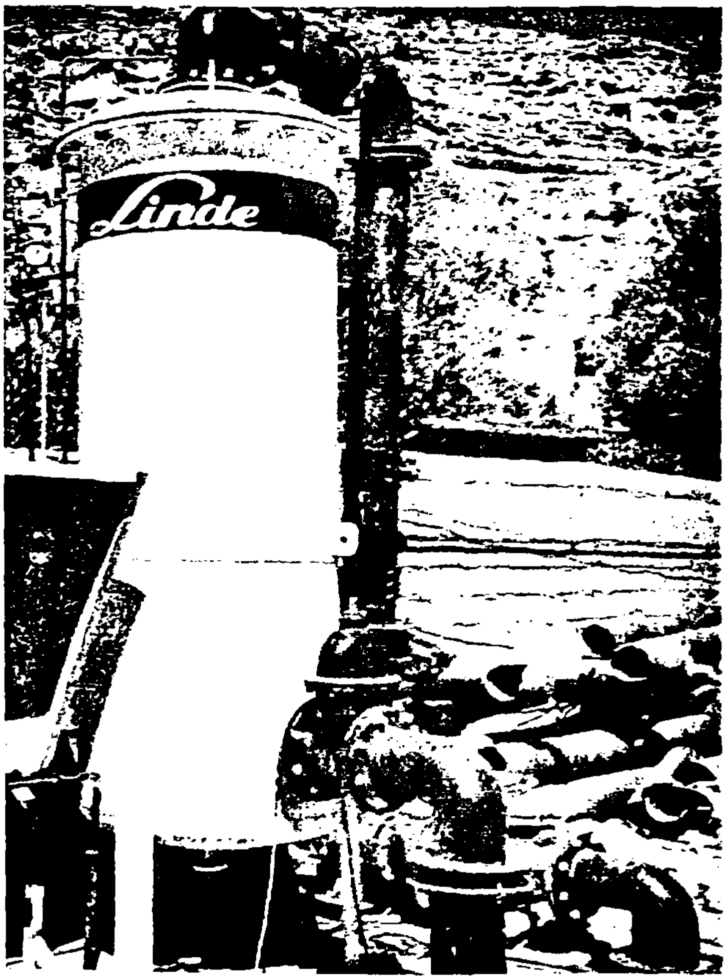

Figure 3.3 : The reactor and it's connections.

reactor. Therefore, the top of reactor was the first point which both gas oxygen and inlet water combined together. Approximately 30cm from the top of the reactor a mesh disk has been fixed. This disk was composed of very small holes. The mixture of water and gas oxygen passed through these small holes and combined very well together. The turbulenec that happened inside the reactor caused the water to be in a very close contact with oxygen, therefore the water inside the reactor became supersaturated. The concentration of oxygen in the water inside the reactor was 40ppm.

The supersaturated water (1/3 of total amount of farm water source) transferred form the reactor to the 7 raceways in Group A. This supersaturated water has been directed from the reactor through a main PVC pipline. Figure 3.4.

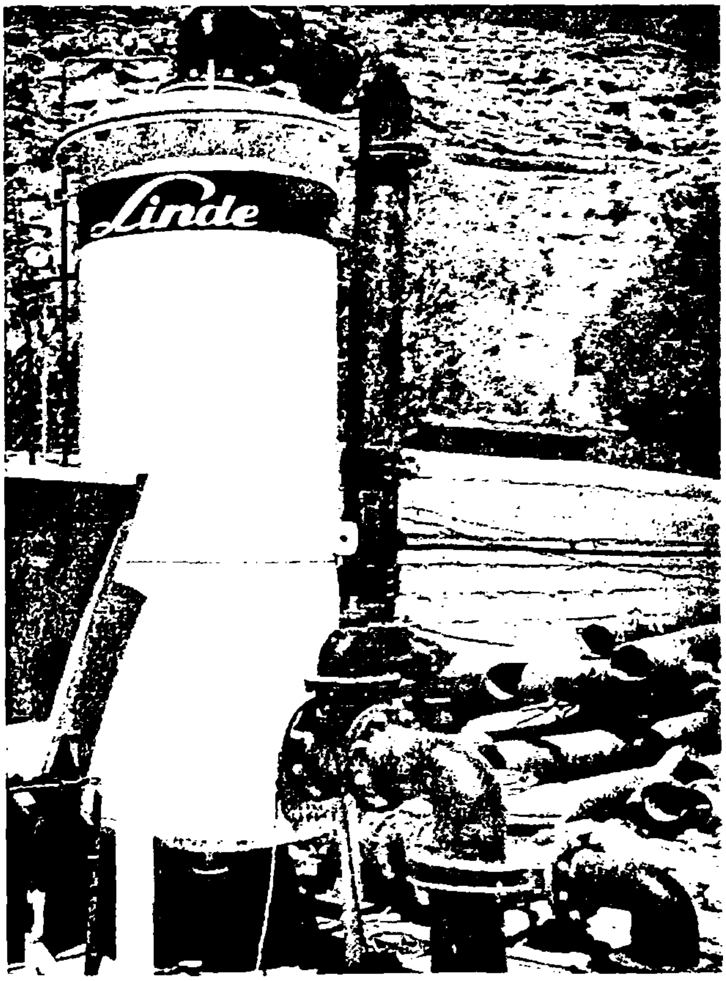

Figure 3.3 : The reactor and it's connections.

The main PVC pipeline subdivided to 7 branches which led to 7 raceways Group A (Figure 3.5).

Each of these 7 raceways in Group A received a specific amount of the oı supersaturated waret from the reactor, depend on the fishes oxygen requirm The amount of inlet supersaturated water to each of the 7 raceways in Grouj has been controlled by a separate valve (Figure 3.6).

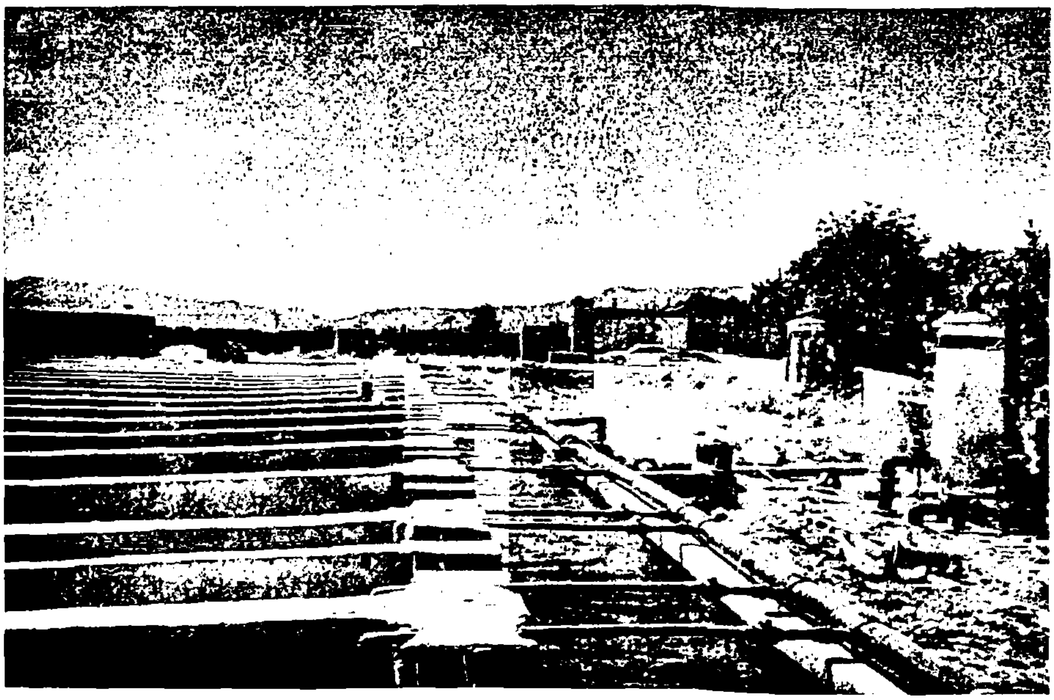

Figure 3.4: PVC pipline and it's subranch to the raceways.

Figure 3.5: The valve which control the amount of supersaturated water in each raceway.

Therefore, the inlet water in each raceways in Group A has been composed 4lit supersaturated water form the reactor with 40ppm oxygen concentrat and 8lit of the main water source of the farm with only 5.5ppm oxy concenrtation. These water with different oxygen concentration inside raceways in Group A combined together. As a result the water in th raceways had 9-10 ppm concentration of oxygen which is an ideal amoun dissolved oxygen to rear rainbow trout.

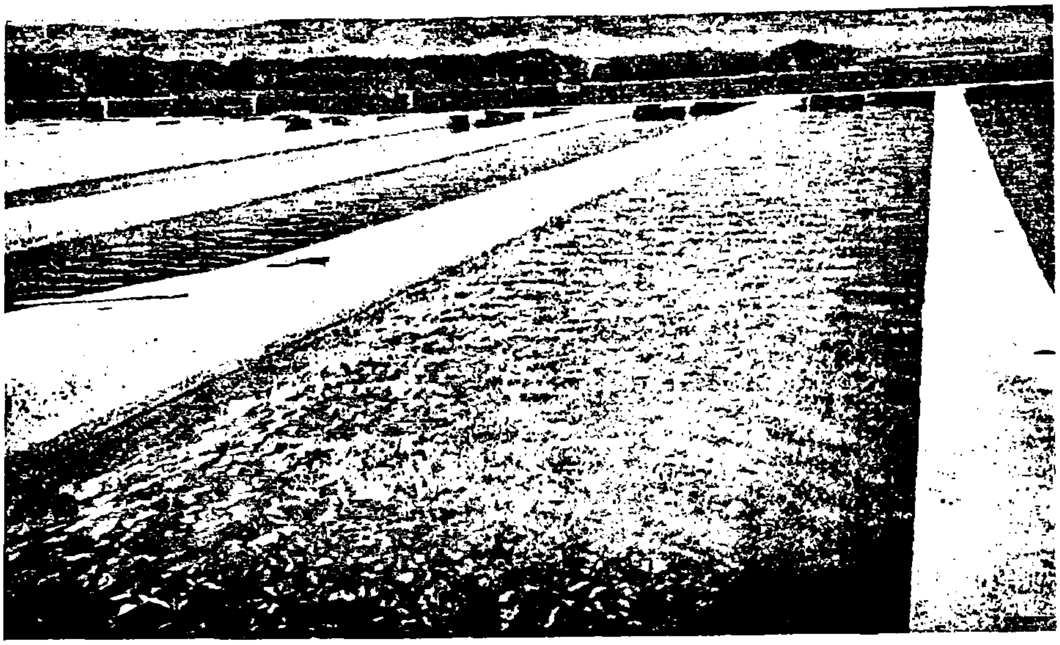

Figure 3.6: Race way of Group A.

On the other hand, inside the 7 raceways in Group B, the only the main wa source of the farm has been directed with 5.5ppm oxygen concentration.

All the physico-chemical paramaters except the dissolved oxyg concentration in raceways of Group A and B were the same.
All the biological charactristic inside raceways in two group were also t same. All the Rainbow trouts were selected from a same brood stock with t homogeneous weight of 30gr per fish.

The consumed fish food during the whole priod of experiment were produc by a local fish feed producer. Fishes has been feed 2 times per day.
The experiment was started on January and last for 6months till the fisl reached the market size (350gr).
During the experiment period the amount of dissolved oxygen in raceways Group A has been checked at least 2 times per day with an oxygen meter. T amont of dissolved oxygen in each raceway has been measured in inlet, in the

middle and in the outlet of each raceway. In a proper condition the amount of dissolved oxygen in inlet of raceway has to be 9 – 10 ppm and in the outlet 5 – 5.5 ppm. If in the reported amount of dissolved oxygen in inlet and outlet of the raceways of Group A was not the same as what was mentioned above, the amount of supersaturated water from the reactor to the raceways of has been changed accordingly. This action has been done either to avoid bublle – gas disease in case of supersaturation of raceway's water or to stop asphyxia if the amount of reported dissolved oxygen in the raceway's water was less than fishes requirment. The rainbow trout has been biosecured by reducing or deacrising the amount of dissolved oxygen in the raceway's water in Group A.

On the other hand, in the raceways of group B, there wasn't any dissolved oxygen security for the fishes which casued many problem during the rearing period.

3.2.4: Experimental Result:

The Rainbow trouts in Group A, had a very good condition, there didn't show any symptom of sickness, asphyxia or bubble gas disease. The growth rate, FCR and generally health condition were acceptable. The fishes have shown toatlly normal behavior, they have swimed in groups and they were sperated in all part of the arceways homogenically. These fishes had consumped all their food.

The last and the most important observation in raceways of Group A was the amount of culturing fish in each m3 of raceways at the end of rearing period. 65kg market size fishes (350gr) in each m3 of raceway was a great record. This means that at the end of production season 20 tons fishes has been harvested from 7 raceways of Group A.

Beside that the fishes have been in a very good condition, the fish flesh was healty and safe for human- begins to consume.

On the other hand in Raceways of Group B a high range of mortality has been observed. This mortality was mostly due to asphyxia. The other factor which caused mortality was accumulation of toxic gases such as NH3 and CO2 in each raceway. As fishes had been stressed due to asphyxia, they couldn't consume their food well, so the food were settled down at the bottom of raceways and increased the amount of toxic gases.

As the amount of dissolved oxygen in raceway's water were very low, all the fishes were gathered in the inlet of raceways to obtain the most amount of availabe oxygen. This gathering caused much more stress for the fishes.

The amount of harvested market size fishes from the raceways of Group B was almost nothing.

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3.3. Nitrogen Experiment in Food Industry In Iran:

Despit the difficulties which we encountered to apply liquid oxygen in fish farm indusrty, since using liquid nitrogen in food industry is supposed to be a common procedure we easily found the chance to observe the result of apply liquid nitrogen in this industry.

3.3.1: Applying Liquid Nitrogen in Food Industry:

We started our experiment in a factory which produced eaidble oil. They had 3 lines for filling the PET bottle of oils. In 2 lines 250 PET bottle per min and in the other line 200 PET bottle per min has been filled by vegetable oil. The PET bottle size was 1lit which containe 900cc equal to 810gr oil. At the top of each PET bottle there was a free space. Depends on the PET bottle shape, the type of oil filling machine and it's specification and the tempreture which oil has been filled the free space size is vary.

In the factory which we started our experiment the size of this free space was 45-50cc.

The free space is actually utalize to apply liquid nitrogen inside the oil bottles. After filling the PET bottle by oil and before capping it the liquid nitrogen has been used (figure 3.7).

In this experiment a cryogentic storage tank has been installed. As in the production line liquid nitogren has been applied, there was no need to install any vaporizer. From crypgenic storage tank a vaccum pipline has been installed till the nitrogen injector injection unit. The liquid nitrogen pressure inside the pipeline was 2bar.

A complete line of liquid nitrogen dosing equipment for entering/nit
ushing and pressurization applications has been applied in this experi
his equipment typically installed after the filler and before the capper,
itrogen injection units dispense a measured amount of LN2. In this stu(
ach PET bottle 1gr liquid nitrogen has been measured.

Figure 3.7: Applying Liquid nitrogen in vegetable oil PET bottle.

Once the liquid nitrogen is introduced into the PET bottles, the cold
itrogen (-196°C) immediately picks up heat and turns into gaseous nitr(
oom temperature and expands rapidly (1 gr of liquid nitrogen yields 85(
itrogen gas) expanding 700 times in the process. When trapped inside tl
ottle, the gaseous nitrogen creates a defined internal pressure in the PE]
and adds rigidity to the PET bottle. By adding a bit more liquid nitrog
easy to increase the internal pressure in the PET bottle, this process helps

manufacturer to use thinner wall PET bottles. These PET bottles with thin wall cans with controlled, high purity liquid nitrogen provides package strength to eliminate paneling and palletizing problems and provides cost savings with the use of lighter weight plastic.

For headspace inserting applications, the rapidly expanding gas is allowed to escape before the product is sealed, flushing oxygen out of the PET bottle. This helps to extend the product shelf life, maintains product taste, color and freshness, reduces oxygen absorption and eliminates paneling.

Application of liquid nitrogen in packing vegetable oil PET bottle has been developed to meet these requirements and cut cost at the same time by providing a precise charge of liquid nitrogen.

3.3.2: Experimental Results:

Controlled, high purity liquid nitrogen dosing in packing vegetable oil line provides:

- Consistent pressure from container to ensure no deformation of container
- Manufacturer cost saving with the use of lighter weight plastic PET bottles.
- Weight reduction of PET bottle.
- Reduction of oxygen content.
- Very low consumption of liquid nitrogen.
- Nitrogen is completely inert and is accepted in the food and

beverage industry. It is totally tasteless and odorless.

- Firm packing for customer appeal.
- Package strength to eliminate paneling palletizing problems.
- Extends product shelf life
- Maintains product taste, color and freshness
- Reduces oxygen absorption by product

CHAPTER 4: CONCLUSION AND DISCUSSION:

In this chapter the conclusion of our experiments in Aquaculture and Food Industry is described. In details we show how oxygen can reduce the risk of introducing pathogens and consequently the stressful conditions in a fish farm. Also, how to enhance the susceptibility of fishes against infection and disease.

In food industry also we concluded the positive effect of applying gases to restore and packing the food products.

4.1: Oxygen – Aquaculture:

By applying oxygen in a fish farm the biosecurity of fishes ensured. In the following segments in details the effect of oxygen in biosecurity of fishes has been described.

4.1.1: Conclusion of the Experiment:

Oxygen is an essential factor in rearing of fish. Applying this gas is a necessary and safe method to rear high population of fishes in a farm with the same facility as before. Therefore a farmer is able to gain more benefit just by adding a little cost to it's own system.

Generally, the successful installation and application of oxygen in the farm and the biosecurity of using it and subsequently the security regulations which applied through this experiment changes the attitude of using gas technology by fish farmer in Iran. More farmers become familiar with this system and it's huge benefit. Therefore, the use of ISO and HACCP systems are necessary if any fish farmer wish to have Such a system we successfully applied in farm A during our experiment.

Beside that as we reared healthy fishes we directly secured the human's health as the consumer of these fishes.

In food industry better maintenance of packed food, design suitable package and applying different gases to secure the health of the food with more shelf life is a great achievement in food industry.

Freezing the food caused an easier life as we can easily access to any kind of the food, anywhere, anytime. The application of gas leads to a great improvement in human's life.

4.1.2.Safety In Aquaculture:

Aquaculture is one of the most vulnerable business areas if problems occur with the gas supply. Production is becoming more intensive and comprehensive, so there is also more risk. The emphasis, therefore, has to be on safety. Should any problems occur with the operation of the tank system, the reaction time is short! And fish mortality will be inevitable caused by suffocation resulting from oxygen injection system failures (even short-term ones) are not uncommon. The oxygen demand in tanks with high densities of large fish is high. During such emergency circumstance, the fish farmer generally has a response time of 10 minutes or less to avoid a total kill. In any case, gas supply from a liquid oxygen tank is the safest way of gas supply available, but it's always advisable to have a backup to cover the worst case. This usually is accomplished by Protection by designing alarm systems or storing some compressed oxygen in cylinders or cylinder bundles for emergencies is essential. This back up as an alternative oxygen supply must be fast connected to the oxygen injection system to prevent any financial ruin.

An emergency water supply reservoir consisting of a large volume of high quality water (oxygenated, dechlorinated) and the proper water temperature stored in a standby tank provides a good margin for safety. Sudden declines in water quality or outbreaks of disease can be effectively controlled in certain instances by rapidly flushing growing tanks with freshwater, by quickly altering water temperatures [44].

Liquid oxygen storage tank must regularly monitor by fish farmer to control the level of available oxygen. Also, an alarm must sets up on Oxygen storage tank in case of tank malfunction due to reducing pressure or any mechanical disorder [46].

To be more secure Liquid oxygen tank must be install in a proper location, and has to be isolate and fenced from the other part of the farm which is reachable by everybody. The storage tank must be installed on a proper designed foundation to reduce the risk of collapsing in case of earthquake.

Smoking near the storage tank or during the delivering Liquid oxygen process from the Iso-container to the liquid storage tank is so dangerous and it is absolutely forbidden.

The gas injection hose must be control to be sure if it work properly and the produces gas bubble is still homogenized. If any malfunction is seen the injection hose must be clean up carefully and set up again.

During the charging of liquid storage tank all the safety of handling hazard with liquid oxygen must be regarded. The main causes of fires and explosions when using liquid oxygen are oxygen enrichment from leaking equipment, use of materials not compatible with oxygen, use of oxygen in equipment not designed for oxygen service and incorrect or careless operation of oxygen equipment which should be considered very carefully.

4.1.3. Biosecurity In Aquaculture:

Often one would like to think that implementing biosecurity practices on the fish farm will prevent entry of even a single pathogen. Realistically, biosecurity for food fish production accomplishes pathogen reduction rather than pathogen elimination.

4.1.4. Application of Biosecurity In Aquaculture:

Biosecurity or "hazard reduction through environmental manipulation" in aquaculture, consists of policies, procedures and measures used to prevent or control the spread of fish disease A biosecurity program (Bebak 1998) is comprised of a variety of practices, policies and procedures used on a farm in order to:

- reduce the risk that pathogens will be introduced to a fish culture facility;
- reduce the risk that pathogens will spread throughout the facility if they are introduced;
- and reduce conditions that are stressful to the fish, which can enhance susceptibility to sub-clinical and clinical disease once pathogen introduction does occur. [47]

Aquaculture biosecurity policies vary from farm-level to the international level, and between areas at each of these levels, but several characteristics are essential if aquaculture biosecurity polices are to be successfully implemented (Scarfe 2003). These common characteristics include: a) science-based decision making, b) economical and sociopolitical rationales, c) standardized and uniform methods, d) relative ease of application, e) wide recognition, f) vertical and horizontal integration, application, and agreement, g) consistent enforcement, and h) a primary focus on prevention, but with contingencies in place for control and management, or eradication. [48]

A biosecurity program should be tailored to the needs of the specific site, while taking into consideration the business needs of the operation, the fish species and life stages grown, and the disease profile of the surrounding region. Overall, a biosecurity program would include, but not be limited to, practices and procedures involving: 1) surveillance for the presence of disease organisms; 2) vaccination; 3) quarantine and restricted access; 3) appropriate practices of fish husbandry; 4) disinfection; and 5) disease treatment (including eradication) [47].

Fish diseases are not now to aquaculture, they continue to be one of the greatest causes of economic loss for the industry. The impact from disease may occur as direct losses due to illness or mortalities as well the indirect effects such as decreased production (e.g., reduced growth rates, feed conversion efficiency, or product quality) or loss of business or reputation. [50]

In the international market of fish and fishery products a major challenge faced by exporters is that different standards and regimes are applied by importing countries on producing countries to ensure that products meet the requirements of the target market. Significant progress has been made in recent decades in improving the standards of post-harvest handling and processing of fish and fishery products with the introduction of food safety management systems based on the principles of the Hazards Analysis and Critical Control Point (HACCP) system. While food safety and hygiene standards have improved in the processing and manufacturing sectors, more still needs to be done to improve such food safety standards in the aquaculture production sector.

In recent years both FAO and WHO have advocated a risk based approach in food safety management and a current priority for both organizations is to promote risk based approaches to food safety management options in aquaculture production. Fish farmers are one of the first links in the food production chain and standards of final products depend on the application of good aquaculture practices being applied on the farm. A major challenge faced by many countries exporting aquaculture products is reducing the incidence of rejection of products because of microbiological contamination. [51]

4.1.4.a. Reducing the risk of Introducing pathogens to a fish facility:

Any food fish production facility that plans to intensify culture in a given water supply, and 1) uses a water supply with a resident population of fish or 2) imports fish into the facility, can expect to experience infectious disease outbreaks if no changes in these two management practices are made. Ideally, a farm would use a pathogen-free water supply that is protected from contamination and would purchase only certified eggs to restock the facility. Unfortunately, not all farms have access to a pathogen-free water supply, nor do all farms culture species that are readily available as eggs. If a pathogen-free water supply is at risk of contamination, or is unavailable, then incoming water should be disinfected.

Ozonation and ultraviolet radiation are the most commonly used methods. If possible, the facility should only be restocked with fish hatched from certified eggs that have been disinfected upon arrival at the facility. If fish must be imported into the facility, then strict quarantine procedures should be implemented. In addition, fish should only be purchased from a reliable source with certified broodstock that has been kept in a pathogen-free and/or disinfected water supply. The risk of pathogen introduction can also be reduced by keeping the number of different suppliers to a minimum. Farms that culture species that are not available as certified eggs should actively support research on broodstock development and egg production.

As biosecurity practices are considered, begin with the areas where the population is most susceptible (e.g., egg and fry rearing areas). Management practices that may be implemented to further reduce the risk of introduction of pathogens include:

- Wash hands with anti-bacterial soap upon entering the facility.
- Disinfect footwear or change footwear to disposable or disinfected nondisposable, boots before entering the facility.
- Access to egg incubation and fry facilities should be restricted to a minimum number of well-trained individuals.
- Reduce the number of visitors to a minimum and/or only people working on the farm should be allowed into the facility.
- Disinfect wheels of delivery vehicles when they come onto the facility and when they leave. Establish a visitor parking area on the periphery of the facility grounds.

4.1.4.b.Reducing the risk that pathogens will spread throughout the facility:

Meticulous husbandry is an essential component of an effective biosecurity plan. Feces, uneaten feed, algae, aquatic plants and other decomposing debris provide a substrate for opportunistic pathogens to flourish. Tank surfaces should be kept free of uneaten feed, feces, algae and aquatic plants. Inflow and outflow pipes, aerators, spray bars and any other equipment inside the tanks should be cleaned frequently.

It is critically important that every part of the rearing system be constructed so that the system can be easily cleaned as necessary. All parts of recycle systems including the biofilters, low head oxygenators and CO2 strippers should be accessible for cleaning. Clean-outs should be installed to access pipe interiors. Construction materials should be non-porous and easy to clean and disinfect. Avoid the use of wood. If wood is to be used, it should be considered disposable. Wood use should be limited to temporary structures and these structures should never be transferred to another site. Culling dead and sick fish is a very important strategy that can reduce the spread of pathogens from fish to fish. How culling will be accomplished should be considered early on in facility design. Culling should be done at least once a day or, if possible, on a continuous basis. Culled live fish should be humanely killed and not allowed to die from suffocation. Monitoring is an important part of early identification, isolation and treatment of a problem. How monitoring will be accomplished should be considered early on in facility development. Ideally, daily observation of the fish should be possible. Dim lighting and very large tanks with limiting viewing access limits the possibility of visual inspection of fish, one of the most valuable tools for detecting an incipient problem. Culled fish should be periodically assayed for pathogens. Records on growth and feed conversion ratios can be used to detect subclinical problems. Consider keeping a susceptible species as sentinel fish.

Other important management practices that will decrease the risk that pathogens will bespread around the facility include:

- Frequent hand-washing with anti-bacterial soap should be standard practice.
- Disinfectant and rinse areas should be readily accessible for disinfecting buckets, nets, dissolved oxygen meters, thermometers and other equipment.
- Tanks and equipment should be disinfected before using for a different group of fish.
- Even when tanks are on the same recycle loop, each tank should be regarded as a discrete rearing unit and the potential for cross-contamination should be minimized.
- Strategically schedule culture activities. Minimize the number of different

personnel working with a particular group of fish. As soon as any suspicious mortality above baseline levels occurs, only one person should be allowed to work with affected fish. Alternatively, if personnel resources are limited, work should be done on the unaffected tanks first, leaving the affected tanks for last.
- Aerosol transmission of pathogens can occur. Consider placing barriers between tanks.
- Minimize transfer of fish between tanks.
- Whenever possible, employ the use of vaccination as a disease

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- Whenever possible, employ the use of vaccination as a disease

prevention management tool.

4.1.4.c. Reduce conditions that are stressful to the fish and that can enhance susceptibility to infection and disease:

Stress associated with crowding, low water flow, poor nutrition, poor water quality and other husbandry related factors will render fish more susceptible to, and aggravate the consequences of, infection with opportunistic and obligate pathogens. There are many strategies that can be used to increase fish vigor and reduce stress. Some of these include:

- Use of gentle fish crowding and other methods of gentle fish handling
- Monitor water quality parameters to verify that they remain within recommended limits.
- Poorly nourished fish are more susceptible to disease. The fish feed schedule and feed characteristics should be such that the fish receive the best nutrition possible.
- Purchase eggs and fish only from optimum year class broodstock.

4.2. Nitrogen – Food Industry:

Applying Liquid Nitrogen for packing vegetable oil was so beneficial for both producer and consumer. As already mentioned the result of our experiment the consumers could receive high standard package with lowers risk of spoiling. The supplier also could sell more product than before.

4.2.1. Food Hazard and Food grade gases regulation:

Hazards are biological, physical, or chemical properties that may cause food to be unsafe for human consumption. The goal of a food safety management system is to control certain factors that lead to out-of-control hazards.

Because many foods are agricultural products and have started their journey to your door as animals and plants raised in the environment, they may contain microscopic organisms. Some of these organisms are pathogens which mean that under the right conditions and in the right numbers, they can make someone who eats them sick. Raw animal foods such as meat, poultry, fish, shellfish, and eggs often carry bacteria, viruses, or parasites that can be harmful to humans.

Food can become contaminated by toxic chemicals or toxins in your establishment or in the environment. Physical objects may also contaminate food and cause injury. Food may become naturally contaminated from the soil in which it is grown or from harvest, storage, or transportation practices. Some foods undergo further processing and at times, despite best efforts, become contaminated. These inherent hazards, along with the hazards that may be introduced in your establishment such as metal fragments from grinding can lead to injury, illness, or death. Hazards are a huge threat to food industry. [52= Food safety America]

HACCP (Hazard Analysis and Critical Control Points) is "a systematic approach to the identification, evaluation, and control of food safety hazards." It is an internationally recognized system used to identify and control Food Safety hazards.

HACCP (Hazard Analysis Critical Control Point) is a concept first used by Pillsbury Company to ensure the safety of food prepared for astronauts in the NASA space program.

HACCP plans are mandatory for the following processes and operations in food Industry:

1. Use of food additives or adding components, a method of food preservation or to render a food so that it is not potentially hazardous.
2. Use of unpasteurized shell eggs in highly susceptible population (HSP) operations to prepare food in quantities other than single service portions.
3. Reduced oxygen packaging (ROP) with barriers.
4. Custom processing animals.
5. Molluscan shellfish tanks.
6. Smoking for preservation.
7. Curing.
8. Time as a public health control.
9. Preventing contamination from hands.

The basis for European food legislation is regulation (EC) No178/2002 of the European Parliament and of The Council of 28 January 2002 laying down the general principles and requirements of food law, establishing the European Food Safety Authority and laying down procedures in matters of food safety. Current and proposed European legislation requires that foods, including gases supplied to the food industry, have to meet increasingly rigorous standards to ensure food safety. Gases are used for a variety of purposes in the food industry, which may include being used as additives, processing aids and ingredients. In particular they have to meet requirements regarding labeling, purity criteria and hygiene.

The particular legislative requirements as they apply to food gases and to offer advice as to how these requirements may be met. These cover legislation in the following areas: use of gases as food additives including purity criteria; hygiene and food safety requirements including use of Hazard Analysis and Critical Control Points (HACCP), labelling and lot marking and traceability.

Among all the different regulation for foodstuffs, we focus on Regulation 1333/2008 on Food additives.

1. Where food additives not intended for sale to the final consumer are sold singly or mixed with each other and/or other food ingredients and/or with other substances added to them, their packaging or containers shall bear the following information:

(a) the name and/or E-number laid down in this Regulation in respect of each food additive or a sales description which includes the name and/or E-number of each food additive;

(b) the statement 'for food' or the statement 'restricted use in food' or a more specific reference to its intended food use;

(c) if necessary, the special conditions of storage and/or use;

(d) a mark identifying the batch or lot;

(e) instructions for use, if the omission thereof would preclude appropriate down the general principles and requirements of food law, establishing the European Food Safety Authority and laying down procedures in matters of food safety. Current and proposed European legislation requires that foods, including gases supplied to the food industry, have to meet increasingly rigorous standards to ensure food safety. Gases are used for a variety of purposes in the food industry, which may include being used as additives, processing aids and ingredients. In particular they have to meet requirements regarding labeling, purity criteria and hygiene.

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(c) if necessary, the special conditions of storage and/or use;

(d) a mark identifying the batch or lot;

(e) instructions for use, if the omission thereof would preclude appropriate use of the food additive;

(f) the name or business name and address of the manufacturer,

(g) an indication of the maximum quantity of each component or group of components subject to quantitative limitation in food and/or appropriate information in clear and easily understandable terms enabling the purchaser to comply with this Regulation or other relevant Community law; where the same limit on quantity applies to a group of components used singly or in combination, the combined percentage maybe given as a single figure; the limit on quantity shall be expressed either numerically or by the quantum satis principle;

(h) the net quantity;

(i) the date of minimum durability or use-by-date;

(j) where relevant, information on a food additive or other substances referred to in this Article and listed in Annex IIIa to Directive 2000/13/EC as regards the indication of the ingredients present in foodstuffs.

2. Where food additives are sold mixed with each other and/or with other food ingredients, their packaging or containers shall bear a list of all ingredients in descending order of their percentage by weight of the total.

3. Where substances (including food additives or other food ingredients) are added to food additives to facilitate their storage, sale, standardisation, dilution or dissolution, their packaging or containers shall bear a list of all such substances in descending order of their percentage by weight of the total.

4. By way of derogation from paragraphs 1, 2 and 3, the information required in paragraph 1 points (e) to (g) and in paragraphs2 and 3 may appear merely on the documents relating to the consignment which are to be supplied with or prior to the delivery, provided that the indication 'not for retail sale' appears on an easily visible part of the packaging or

use of the food additive;

(f) the name or business name and address of the manufacturer,

(g) an indication of the maximum quantity of each component or group of components subject to quantitative limitation in food and/or appropriate information in clear and easily understandable terms enabling the purchaser to comply with this Regulation or other relevant Community law; where the same limit on quantity applies to a group of components used singly or in combination, the combined percentage maybe given as a single figure; the limit on quantity shall be expressed either numerically or by the quantum satis principle;

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container of the production question.

5. By way of derogation from paragraphs 1, 2 and 3, where food additives are supplied in tankers, all of the information may appear merely on the accompanying documents relating to the consignment which are to be supplied with the delivery [50].

4.3. Discussion:

By increasing the living condition of fishes the following result were concluded. Due to the optimum amount of dissolved oxygen in water, the fishes could eat more. As a result the growth rate of the fish population increase 20-40% depending on the fish origin, the fish resistances against disease increase by 10-50%. We gain 40% more fish biomass in the same pools in compare the previous raring period.

In food industry also by increasing the packing condition of edible oil bottles, the transportation, package form, shelf-life of oil inside the bottle increase significantly.

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