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CO-OPERATIVE WORKTERMS REPORT

PRESENTED

BY

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Preface

This report were created during the author was doing training in food science department, University of Guelph, Ontario, Canada in the area of Food Microbiology , Co-operative student program during June 3 to Dec 20, 1996. Most of informations involve about job description, organization of department, the results of work term co-operative, they include experience which the author got during that period. The author think that it will be useful for someone who is interested in Modern technology in Food Microbiology and for the co-operative students of the next generation to gain ideas about the co-operative program.

Thank you The HRD SUT-CUTC Project (co-operative education student exchange program) so much for giving me a good chance to come to Canada.

Podjana Chumkhunthod
Dec 20,1996

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1. The organization of Food Science Department University of Guelph, Ontario, Canada.

Chair--Marc LeMaguer

Graduate co-ordinator--Rickey Y.Yada

Graduate secretary--Margaret Walmsley

Graduate Faculty:

Shai Barbut--Associate Professor

Douglas G. Dalgleish--Professor and Ontario Dairy Council and Industrial Research Chair in Dairy Technology

H. Douglas Goff--Associate Professor

Mansel W.Griffiths--Professor and Ontario Milk Marketing Board and Industrial Research Chair in Dairy Microbiology

Schraft Heidi--Associate Professor

Linda J.Harris--Associate Professor

Arthur R.Hillnd--Associate Professor

Yukio Kakuda--Associate Professor

Marc LeMaguer--Professor

Robert W.J.Lencki--Assistant Professor

Alejandro G.Marangoni--Assistant Professor

Ronald E.Subden--Professor

Howard J.Swatland--Professor

Marvin A.Tung--Professor and Weston Foundation Food Packaging Technology Chair

Rickey Y.Yada--Professor

Associated Graduate Faculty:

Jose M.Aguilera--Professor, Chemical Engineering, Universidad Catolica de Chile,adjunct Professor

Norman Ball--University of Waterloo

Malcolm C.Bourne--Professor, Food Science and Technology, Cornell University

Carole Buteau

Robert C.Clarke--Health of Animals Laboratory,Agriculture Canada Guelph

Robert H.Coffin--Cavendish farms,Summerside P.E.I.,Adjunct Professor

Valerie J. Davidson--Adjunct professor

Christopher J.Findlay--Compusense Inc., Guelph

Beniot Girard--research Scientist, Agriculture and Agri-Food Canada, Summerland, BC

Elizabelt A.K. Gullett--Adjunct Professor

Michael J.Hincks--ORTECH International, Mississauga
 Herbert O.Hultin--University of Massachusetts marine
 Station,Gloucester
 Basil S.Kamel--Vittoria, Ontario
 Robin C.Mackellar--Agriculture and Agri food Canada, Ottawa
 H. Wayne Modler--Agriculture and Agri Food Canada, Ottawa
 Vladimir F.Rasper--Professor Emeritus and Adjunct Professor
 David W. Stanley--Adjuct Professor
 Samuel Wang--Vineland Research Station Adjunct Professor
 O.P. Ward--University of Waterloo, Adjunct professor

1.1 Organization Chart of Food Science Deparment University of Guelph, Ontario, Canada

```

Food Science----->
    .----->Dairy: Mansel W. Griffiths
    .                  Douglas G. Dalglish
    .                  Arthur R. Hill
    .                  H. Douglas Goff
    .
    .----->Meat: Shai Barbut
    .                  Howard J. Swatland
    .
    .----->Microbiology: Mansel W. Griffiths
    .                      Schraft Heidi
    .                      Ronald E. Subden
    .
    .----->Chemistry: Alejandro G. Marangoni
    .                      Rickey Y.Yada
    .                      Yukio Kakuda
    .
    .----->Physic/Material Science:
    .                      DouglasG.Dalglish
    .                      H. Douglas Goff
    .                      Marvin A. Tung
    .                      David W. Stanley
    .
    .----->Engineering: Marc LeMaguer
    .                      Robert W.J. Lencki
    .                      Valerie J. Davidson
  
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1.2 Coworkers in Food Microbiology Laboratory

Dr. Mansel Griffiths-Professor

Dr. Heidi Schraft-Assistant Professor

Dr. Jinru Chen-Research Association

Ph.D Students

- Lynn McIntyre
- Derrick Bautista
- Reem Barakat

M.Sc. Students

- Stacy Favrin
- Andy Buechin
- Stephane Cadieux
- Shirley Lin

Undergraduate Research Student

- Suzanne Smith

Technician

- Yolanda Hirvi
- Ann Toner
- Sukhvinder Kaur

International Co-operative students

- Podjana Chumkhunthod from Thailand
- Hedwig Kasstra from Netherland
- Bianca Brunke from Germany

2. Outline of co-op workterm project

Main Project

"Rapid Monitoring Method to Assess Efficacy of Sanitizers against *Pseudomonas putida* Biofilms"

Part I Biofilm Production.

Part II Evaluation efficiency of 2 biocides to damage biofilm.

Special experiment

"Use of molecular biology to confirm the locations of the lux gene on the bacterial DNA chromosome."

consist of:

- DNA extraction
- Agarose gel electrophoresis
- RNA digestion
- Restriction of DNA
- Southern Blot Membrane
- Southern Hybridization
- Detection of DIG-labeled Nucleic Acids

.....

2.1 Biofilm Production

-Test cultures

Five luminescent clone strains of *Pseudomonas putida* were used in this experiment.

objectives:

1. Identify clones with high luminescence and low luminescence.
2. Relate RLU (relative light units) reading to actual cell number by using luminometer and total viable count.
3. Make correlation curve by using Quattro Pro (computer program).

-Biofilm formation

objectives:

1. Try to produce biofilm on rubber gaskets.
2. Test efficiency of 3 different media for biofilm formation.
3. Previous information about clones.

Several methods are used to monitor and check biofilm formation.

- Vortex and ultrasound
- Luminometer
- Epifluorescence microscopy (EFM)
- Scanning electron microscopy (SEM)
- Viable count
- Bactometer
- CCD (charge coupled device)

2.2 Evaluation efficacy of 2 sanitizer to damage biofilm objectives:

1. Test efficacy of sanitizers commonly used in dairy plant to biofilm of *Pseudomonas putida*.
2. Perform using bioluminescence for rapid microorganism monitoring to access efficacy of sanitizing compare with traditional plate count.

Two types of sanitizer are used for treatment.

- Non-foaming Acidic sanitizer (Divosan Activ)
- Liquid hypochlorite sanitizer (Dibac)

Monitoring methods

1. Bioluminescence
2. Aerobic plate count. In this case, Spiral plating are available.

2.3 Special experiment

Objectives:

1. Try to extract chromosomal DNA from Bioluminescence strains of *Pseudomonas putida*.
2. Isolate and Purify chromosomal DNA and confirm by Agarose Gel Electrophoresis.
3. Identify location of lux AB on chromosomal DNA by Southern Hybridization.
4. Previous Correlation of Lux gene location with Biofilm Production.

3. Rapid Monitoring Method to Assess Efficacy of Sanitizers against *Pseudomonas putida* Biofilms.

3.1 Abstract

Biofilms of luminescent *Pseudomonas putida* were developed on rubber surfaces by incubation in brain heart (BHI) infusion broth. Scanning electron microscopy (SEM) and Epifluorescence microscope (EFM) were used to examine biofilm formation. To test efficacy of two sanitizers commonly employed in dairy plants for CIP (Clean In Place) procedures, bioluminescence and aerobic plating were used to enumerate cell numbers. Immediately after the sanitizer treatments, an apparent 5 log reduction of biofilm associated cells was determined. However, when the samples were resuscitated for 18 h in BHI broth, high numbers of cells were detected which reached levels close to those of non treated controls. Results demonstrated that neither sanitizer could completely eliminate biofilm associated *P. putida*. The novel method, microbial bioluminescence, proved to be the best way for assessing efficiency of sanitizers against microbial biofilms.

Keywords: *Pseudomonas putida*, bioluminescence, biofilm, sanitizers.

3.2 Introduction

Bacterial attachment to solid surfaces and biofilm formation are growing to be a serious concern in the food industry (27). It is recognized that bacterial adhesion can be a potential source for food contamination affecting quality and safety of products (3, 16). Biofilms can be formed on a variety of materials commonly used in food plants. These food contact surfaces include glass, stainless steel, polypropylene, rubber and polytetrafluoroethylene (2, 3, 16, 21, 22, 24, 29). Although these materials appear to be smooth, they may have crevices or small cracks that could harbor bacteria which may shed into the food processing environment (3, 21). It has been documented that biofilm cells have a higher resistance to antimicrobial agents than bacteria in culture suspensions because they are embedded in extracellular substances which protect cells from direct sanitizer exposure. Thus, bacteria may survive on the surface of equipments after cleaning and sanitizing (8, 17, 24, 30). To assess efficiency of sanitizers against biofilms, bacterial

bioluminescence could be a great choice to monitor survival of microorganisms (28). Bioluminescence is closely related to the basic metabolism of bacterial cells. It involves reducing a flavin mononucleotide which is catalyzed by the enzyme luciferase that is responsible for light emission (4, 10, 20). This reaction needs a long chain fatty acid aldehyde as substrate. Bacteria can be transformed to a luminescent phenotype by using genetic engineering to insert the luciferase gene (*luxAB*). The amount of light emitted by such bacteria is directly proportional to cell numbers and can be rapidly monitored by a luminometer. Many studies have demonstrated that the most dominant genus of the psychrotrophic microflora present in milk processing plants is *Pseudomonas* which is well known as a biofilm producer. In this case study, bioluminescent *P. putida* were used to form biofilms and subsequently treated with sanitizers commonly employed in dairy plants. Efficiency of the sanitizer treatments was evaluated by traditional plate counting and by using the novel method of bioluminescence. The goals of the study were i) evaluation of the efficacy of sanitizers commonly used in dairy plants against microbial biofilms, ii) to examine bacterial attachment of luminescent *P. putida* on Butyl rubber surfaces, iii) to compare advantages and disadvantages of plate counts and bioluminescence as enumeration methods and iv) to examine effect of post treatment resuscitation on the detection of surviving bacteria.

3.3 Materials and Methods

3.3.1 Bacterial cultures

Pseudomonas putida LV 2-4, which had been isolated from biofilm in a milk processing line by Dr. J. Austin, Bureau of Microbial Hazards, Ottawa was used in this study. To obtain a luminescent phenotype, *luxAB* was introduced into the chromosome of *P. putida* LV 2-4 by using the plasmid PT 7-5 according to the procedures described by Chen and Griffiths (5a). One clone, emitting a strong bioluminescent signal while still maintaining its ability to form biofilms was used in this study. Cultures were grown in brain heart infusion (BHI) broth at 30°C overnight.

3.3.2 Biofilm Formation

One mL of an overnight culture of *P. putida* LV 2-4-lux was inoculated into a sterilized flask containing 10 mL of BHI broth

and 3 rubber septa (Butyl rubber/polytetrafluoroethylene, materials commonly used in dairy plants). Inoculated flasks were incubated at 22°C with 70 rpm shaking. Every 12 h the growth medium was replaced with 10 mL of fresh BHI broth (22). After 1, 2, and 3 days, samples were aseptically removed from the flasks and treated with various sanitizers.

Biofilm formation was verified using scanning electron microscopy (SEM), epifluorescence microscopy (EFM) and enumeration by plating after removal of bacteria from the surfaces. For SEM, the sample surfaces were prepared by fixing the biofilm cells with standard buffer (0.07 M KH_2PO_4 , 0.07 M Na_2HPO_4) containing 2% glutaraldehyde overnight at room temperature. Samples were then rinsed for 10 min. in standard buffer and dehydrated in following an ethanol series. Dehydration was completed by critical control point drying with CO_2 . Samples were coated with Au/Pd 30nm with a Hummer VII sputter coater (Anatech JTD Alexandria, VA) and viewed in a scanning electron microscope (Hitachi S-570, Tokyo, Japan) at 50 KV.

For EFM the samples were stained with 1% acridine orange for 1 min. At room temperature and mounted onto a microscope slide. Attached cells were viewed in an epifluorescent microscope with a 100x oil immersion objective.

3.3.3 Enumeration of bacteria

Viable cell counts were determined by surface plating on BHI agar using a spiral plater (Spiral systems). Incubation was at 30°C for 24, 48 and 72 h. Bioluminescence which has a linear correlation to viable cell counts was determined as follows: One mL of the bacterial suspension was thoroughly mixed with 10 μL of 1% n-decanal and light emission was determined in a luminometer.

3.3.4 Effects of sanitizer treatment on biofilms

Two types of sanitizers obtained from Diversey Inc. (Mississauga, ON) were tested: i) a non foaming acidic sanitizer (Divosan Activ) and a liquid hypochlorite sanitizer (Dibac). Working solutions were prepared according to the directions of the manufacture. Phosphate buffered saline (PBS [0.14M NaCl, 0.003M KCl, 0.01M Na_2HPO_4 and 0.002M KH_2PO_4]) + 2% Tween 80, was used as neutralizer for Divosan Activ. The hypochlorite sanitizer (Dibac) was neutralized with 0.01 M sodium thiosulfate. At the end of the incubation period, each rubber septum was

aseptically removed from the culture flask and rinsed 10 times in 50 ml of PBS to remove any unattached cells. After rinsing, the rubber septa were cut in 4 sections by a sterile scalpel and each segment was immersed in 10 ml of sanitizer solution for 30 sec at 25°C. Activity of the sanitizer was stopped in neutralizing solution for 5 min. Then the samples were transferred into PBS with 2% Tween 80 solution and biofilm cells were removed from the surface by vortexing for 2 min at maximum speed. The number of viable cells was determined in each sample before and after vortexing by spiral plates and luminometer. To evaluate survival of injured cells, the entire sample after vortexing (rubber septa and PBS+Tween 80 suspension) was transferred into 5 ml of BHI broth and incubated at 30°C. After 2 and 18 h bioluminescence of resuscitated cells was determined. For control samples, sanitizer and neutralizing solutions were replaced with PBS; otherwise treatments were performed identical to the test samples.

3.4 Results

3.4.1 Biofilm formation

Bioluminescent *P. putida* grew well in BHI broth at 30°C and 22°C. Growth rates were identical for the parent and the bioluminescent strain. The cell numbers necessary to detect a positive luminescence signal were at 10^4 CFU. Preliminary experiments using a variety of culture media and conditions had shown that incubation at 22°C with shaking and regularly replacing the culture medium improved biofilm formation (data not shown). Formation of biofilms on the surface of the rubber septa after incubation for 1, 2 and 3 days was visually confirmed by EFM and SEM (Figure 1). Cells present on the rubber septa which had not been removed by rinsing were considered to be adherent. These adherent cells were readily removed by vortexing with PBS+Tween 80 for 2 min. Quantification of attached cells, determined by subtracting the cell numbers before vortexing from the number of cells detected after vortexing, showed the number of biofilm associated bacteria to be between 10^4 and 10^5 CFU/cm². Accordingly, the value of light emission was between 2 and 200 RLU /cm². The number of cells adhering to the surfaces did not significantly change over a 24 to 72 h period. Different ages of biofilms did not result in different numbers of attached cells.

3.4.2 Sanitizer treatment

After biofilm cells were exposed to two types of commercial sanitizers with the maximum concentration recommended by the chemical manufacturer for 30 sec contact time, biofilm associated bacteria were reduced by more than 5 log cycles. No apparent differences in cell reduction were observed for 1-day, 2-day or 3-day old biofilms. There was no visible difference between sanitizer efficiency assessed with CFU or RLU. This would indicate that the sanitizers were effective for eliminating the biofilm associated *P. putida*. However, after the treated biofilm surfaces were resuscitated in fresh media of BHI and incubated at 37°C by shaking for 2 h, luminescence was again detectable. This light emission increased markedly after a prolonged incubation of the samples for 18 h. The RLU values increased to almost as high numbers as the non treated biofilm controls. Different ages of *P. putida* biofilms did not give different results and no marked difference in efficacy was observed between the non-foaming acidic sanitizer and the liquid hypochlorite sanitizer.

Figure 1: Scanning Electron Microphotograph of *P. putida* biofilm after 1 day incubation

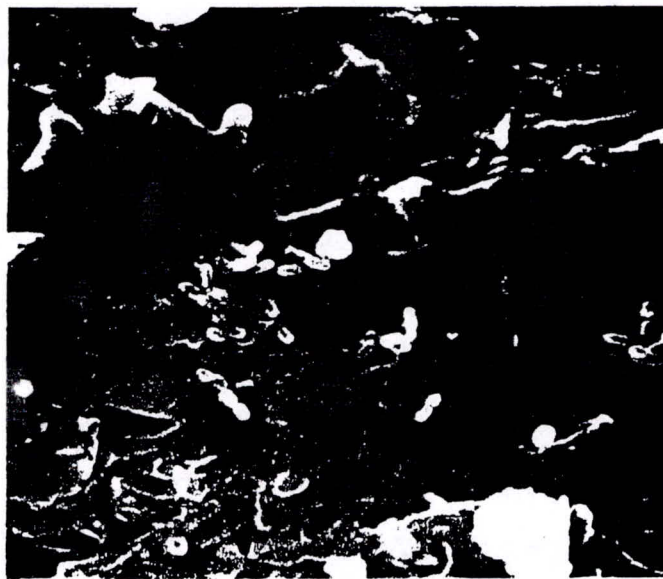


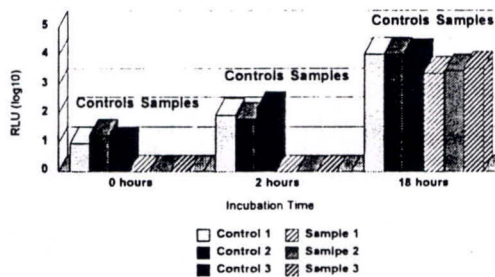
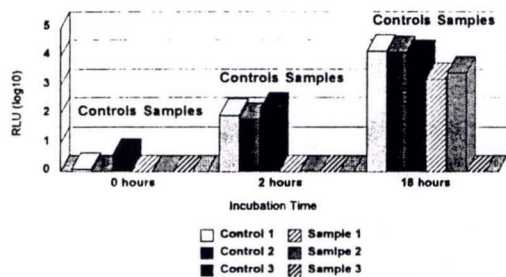
Figure 2: Effect of two commercial sanitizers against biofilm associated *Pseudomonas putida*

Non-Foaming Acidic Sanitizer

Liquid Hypochlorite Sanitizer

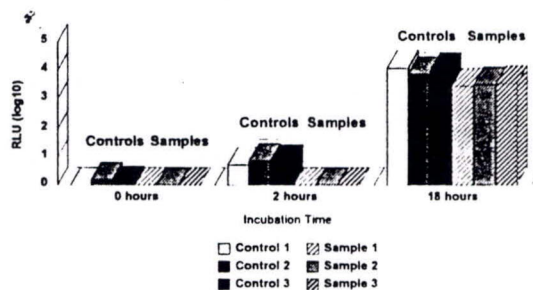
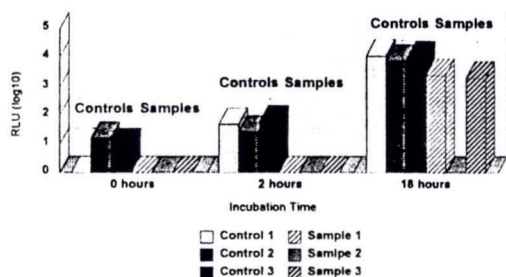
Biofilm 1 day old

Biofilm 1 day old



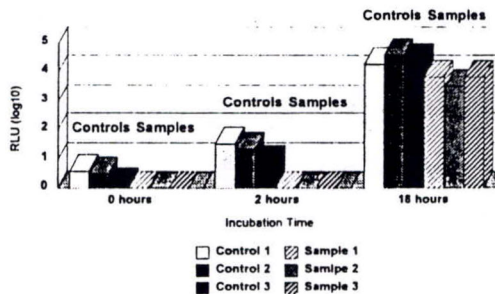
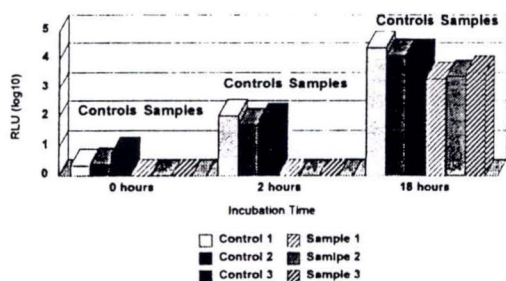
Biofilm 2 days old

Biofilm 2 days old



Biofilm 3 days old

Biofilm 3 days old



3.5 Discussion.

The bacterial strain used in this study was obtained directly from a milk processing plant. Thus, the population of *P. putida* used could be considered a significant source of milk contamination. This research demonstrated that under circumstances close to those in food processing plants, *P. putida* can grow and form biofilms on rubber surfaces within 24 h.

Surface irregularities such as roughness, crevices and pits have been shown to increase bacterial adherence by increasing cell attachment and reducing the ability to remove cells (1). In our study, we chose butyl rubber as contact surface. Visualisation of biofilm by SEM illustrated the original rubber surface displayed a lot of crevices and small holes. In these regions most attached cells were found. It was wondering that very little of extracellular matrix (ECM) which believed to be polysaccharide in nature that may protect cells from antibacterial agents appeared. (24) It was possible that BHI broth are not rich media. Cell were starved and decreased polysaccharide production or that there were many porous on rubber surface which made it easy for cells attached so it was not necessary to produce much ECM aid for adhesion. The other way was used to viewed biofilm formation, is epifluorescence microscope.

Although Scanning electron microscope and epifluorescence were able use for confirm biofilm formation, visual biofilm structure or the presence of ECM. But they did not give quantitative results. So Spiral plating system was investigated for enumeration in this case study. However, viable plate count takes a long time and needs labour. This work we tried to use bioluminescence for assessing efficacy of sanitizer treatment too. Luminometer was used compare with spiral plate count. Results indicated that bioluminescence had more efficiency than traditional plating because it needed only short time for whole procedure, did not need much labour and could used to measure while there were only small amount of sample left. Beside that, we found that there were some common problem from traditional plating. One was arised when cell were removed from surface is that large clumps. It might be not dispered. This leded to be a source of error because cells cound not form colony on the agar surface. It was difficult to count accurately cells and the total number of cells might be unestimated. (13) The other point, Our study were unable to get accuratly results from enumeration by spiral plating in rescusitation step more than bioluminescence

method because there were injured cells unable to grow on BHI agar but still alived.

Results of works conducted by many researchers suggested that the resistance of microorganisms were affected by the ages of biofilm (3, 31). This study 1-day, 2-day and 3-day old biofilms were tested for sanitizer treatment with two commercial sanitizer. After 30 sec exposure time, the results of measuring showed that no different marks of efficiency between non forming acidic and liquid hypochlorite sanitizer on various ages of biofilm, probably the attachment and biofilm development were different in processes and culture.

In conclusion, This work has shown that (a) the behavior of biofilm cells in extracellular production was affected by the type of surface and nutrition media. (b) This research supposed with the earlier reports that the sanitizer efficacy was low in porous surfaces such as rubbers. (c) Engineering a lux gene construction into food isolated of *Pseudomonas putida* provided rapid monitoring method for assessing efficiency of sanitizer treatment. (d) Both sanitizers seemed to be effective to kill bacteria adherence after treatment but after injured were enriched with fresh media, they can survived and reproduced themself quickly. So we concluded that non forming acidic and liquid hypochlorite sanitizer could not eliminate the biofilm associated *Pseudomonas putida* cells.

It's difficult to remove the biofilms completely from the surface and regular cleaning and sanitizing still be the best way to prevent biofilm. So the frequency and adequacy times of sanitizing should be important feasible point for controlling microbial attach in food processing plant.

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4. Special Experiment

"Use of molecular biology to confirm the location of the lux AB on the bacterial chromosome"

4.1 Objectives

1. Learn about molecular biology
2. Perform several steps to identify the location of lux gene of *Pseudomonas putida* in clone 4-8.
 - 2.1 DNA extraction
 - 2.2 DNA detection by Gel electrophoresis
 - 2.3 RNA digestion
 - 2.4 Restriction of DNA
 - 2.5 Southern transfer
 - 2.6 Southern hybridization
 - 2.7 Detection of DIG-labeled nucleic acids
3. Previous information of each clone.
4. Conclusion correlation of location of lux gene with biofilm production.

4.1.1 DNA Extraction

objective : Extract chromosomal DNA from Microorganism.

Materials & methods

- Bacteria clones 4-8 of *P. putida* are cultured in BHI broth to be sampled
- Take 6-10 ml of bacteria culture in centrifuge tubes and centrifuge at 5 degree c for 15 min.
- Suspend pellet in 800 ml of 6.7% sucrose by vortexing.
- Add 50 microlites of lysozyme (10 mg/ml and shake at 37 degree c for 30 min).
- Add 10 microlites of proteinase K (20 mg/ml and shake at 37 degree c for 30 min).
- Add 1 ml of Phenol/Chloroform/Isoamyl alcohol (ratio, 25:24:1) underfumehood, shake quickly and then spin at full speed for 15 min by centrifuge at 5 degree c. After the first spin, the supernatant solution is collected.
- Add 1ml of Phenol/CH₃Cl/Isoamyl (mix together).
- Spin full speed at 4 degree c for 15 min.
- 500 microlites are collected and transferred to lock safe eppendorf vials.
- Add 50 microlites of Na-Acetate, pH 5.2 (0.1*volume of last volume).
- Add chilled 100% ethanol (2 times of sample).
- Store overnight at -20 degree c
- Check for precipitate, spin down at maximum speed for 15 min at

- 4 degree c.
- Discard the supernatant
- Wash with 70% ethanol and spin again for 15 min, allow the pellet to dry. Then the pellet is resuspended in TE buffer, pH 7.6.
- Put it in the oven to dissolve for 1-2 hours at 42 degree c.
- Keep it in the fridge.

4.1.2 Agarose gel electrophoresis

Objective: Verify presence of chromosomal DNA.

Materials & Methods

- Agarose gel preparation (0.8% agarose in 1*TAE buffer)
- Add Ethidium bromide (10 mg/ml) and boil until it becomes clear.
- Cool down and pour in the gel chamber (make sure to set the comb ready).
- Immerse the gel in the chamber and cover with 1*Tris-Acetate-EDTA buffer (TAE buffer).
- Drop DNA sample (mixing solution of 2 microlites of loading buffer, 2 microlites of sample and 5 microlites of distilled water).
- Run the gel at 50-100 volts for 45 min.
- Keep the remaining sample in the fridge for RNA digestion.

4.1.3 RNA digestion

Objective: Digest RNA

Materials & Methods

- Add 0.8 microlites of RNase in thawed sample and spin a little bit.
- Incubate at 37 degree for 2-3 hours.
- Keep at room temperature.
- Run gel electrophoresis for to verify complete digestion of RNA.

4.1.4 DNA restriction

Objective: Cut DNA chromosome into fragments

Materials & Methods

- Add 2 microlites of restriction enzyme (EcoRI), 1.5 microlites of buffer solution and 1.5 microlites of distilled water into tube containing 10 microlites of chromosomal DNA.
- Incubate at 37 degree c overnight
- Prepare agarose gel of appropriate composition (0.8%) a high purity nucleic acid grade agarose and a Tris-Acetate-EDTA

(TAE) buffer.

- Run the gel. If desired, the gel may be stained with Ethidium bromide to visualize the DNA fragments and confirm subsequent Southern transfer to membrane.

4.1.5 Southern transfer

Objective: Transfer chromosomal DNA fragments from the gel to membrane.

Materials & Methods

- Depurinate for 5 min by submerging the agarose gel in 250 mM HCL while shaking at room temperature.
- Rinse the gel with distilled water.
- Submerge the agarose gel in denaturation solution for 45 min at room temperature. Shake gently. This incubation denatures the DNA target prior to transfer.
- Rinse the gel with distilled water.
- Submerge the gel in neutralization solution twice for 15 min at room temperature to neutralize the gel.
- Blot the DNA from the gel by capillary transfer to the membrane (Nylon membrane), using 10*SSC buffer.
- Change tissue after first 1-2 hours before leaving it overnight at room temperature.
- Rinse membrane briefly with distilled water.

DNA fixation

Objective: To fix the DNA on the membrane.

Materials & Methods

- DNA is efficiently bound to the membrane by UV-crosslinking for 5 min.
- The membrane can be used immediately for hybridization. In this case, it was stored dry at room temperature for future use.

4.1.6 Southern Hybridization

Objective: Hybridize DIG-labeled probe with immobilized target nucleic acid.

Materials & Methods

Prehybridization

- Place the blot in roller tubes containing 20 ml standard prehybridization solution and incubate in hybridization oven at 60 degree c for 1-2 hours.
- Heat probe in boiling water for 10 min in order to denature the DNA and chill immediately on ice.
- Discard the prehybridization solution out.

Hybridization

- Add the hybridization solution containing the DIG-labeled probe and allow the probe to hybridize overnight at 60 degree c.
- At the end of Hybridization, pour the hybridization solution from the roller tube into a capped tube. Store the tube at -20 degree c. Hybridization solution can be reused several times.
- Wash the membrane twice, 5 min per wash, in 2x wash solution at room temperature to remove unbound probe.
- Wash the membrane twice, 15 min per wash, in 0.1x wash solution at hybridization temperature (long probe, >100 bp should be washed at 68 degree c).

4.1.7 Detection of DIG-labeled nucleic acids

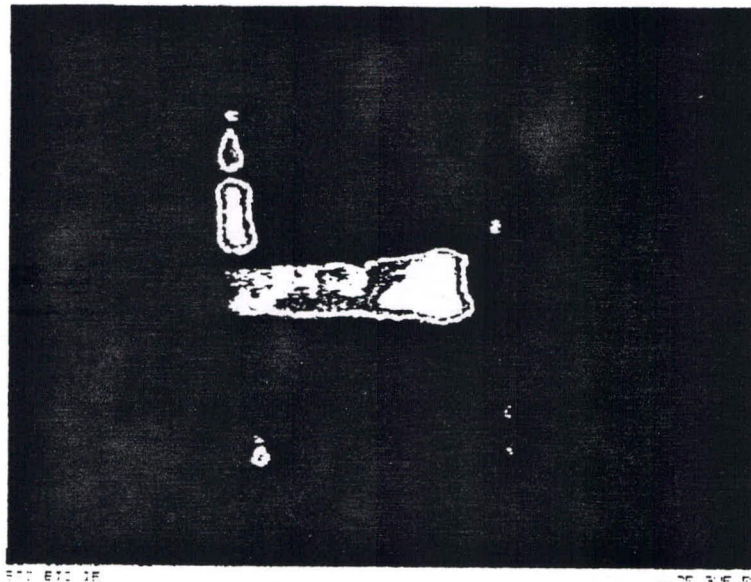
Objective: To detect DIG-labeled Nucleic acids.

Materials & Methods

- After hybridization and Post hybridization wash, equilibrate the membrane in buffer 1 for 1 min.
- Block the membrane by gently agitating in buffer 2 for 30-60 min.
- Incubate for 30 min in Antibody solution (containing 10 ml of 1% Blocking and 2 microlites antibody fragments)
- Discard the antibody solution twice for 15 min in buffer 1 (buffer 1 plus 0.3% Tween 20).
- Pour buffer 1 off and equilibrate the membrane in buffer 3 for 5 min
- Add lumigen PPD 5' (dilute 1:100 in buffer 3), wet membrane thoroughly for 5 min.
- Incubate in plastic seal at 37 degree c for 10 min.
- For detection, the membrane is measured by charge coupled device (CCD) camera.

4.2 Results and Discussion

After membrane was visualized by charge coupled device (CCD), the location of lux AB were performed. For each clone the location of lux gene are different spot. They were shown in picture 1.



clone 4
clone 5
clone 6
clone 7
clone 8

Almost every clones were able identify the location of lux AB in chromosomal DNA. Except clone 7, the location was not good performed. It is possible that supernatant collection was not done completely in DNA extraction step so there wer some chromosomal DNA got lost. There are small amount left. When it was detected with DIG-labeled nucleic acids. Low amount of light emission appeared by CCD camera.

4.3 Conclusion

- The location of the lux AB on chromosomal DNA were able to express.
- Each clone of *P. putida* (clone 4,5,6,7,8) have different location of lux AB.

4.4 Reference

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5. Summery all activities during work term.

- 5.1 Working in Laboratory for main project and special project every day.
- 5.2 Participate in Laboratory Meeting every two weeks and in the lab party each times.
- 5.3 Presentation of project in Laboratory Meeting.
- 5.4 Volunteer in Food Science Conference, Guelph, Ontario, Canada.
- 5.5 Traveling to Montreal, Quebec and Boston with co-workers from the Labolatory as a vacation.
- 5.6 Traveling around Ontario during Holidays.

Advantages

- Learning more culture and traditional of other country especially Canada.
- Improving adjustment for new surrounding
- Learning more about laboratory technique.
- Solving various problems with myself.
- Developing idea and knowledge in various areas.
- Improving English language.
- Learning more about using libraly such as using libraly database computer search the specific information.

6. Recommendation for co-op program.

International trainee student should have international student identification card to identify themself.

ACKNOWLEDGEMENT

Thanks you Dr.Mansel Griffith so much for accepting me to be trainee student in his laboratory and support everthing for all project.

Thanks Dr. Heidi Schraft for everthing her teaching and training all the time I was in Canada.

Thank you very much
The author

Appendix

Solutions required for Southern Blotting are listed below. Refer to Appendix C for details on preparing the additionally required solutions.

Solutions	Description
HCl	250 mM HCl.
H ₂ O	Sterile, distilled water.
Denaturation solution 1	0.5 N NaOH, 1.5 M NaCl.
Neutralization solution 1	0.5 M Tris-HCl, pH 7.5; 3 M NaCl.
20 x SSC buffer	3 M NaCl, 300 mM sodium citrate; pH 7.0.
5 x SSC buffer	750 mM NaCl, 75 mM sodium citrate, pH 7.0.
Standard prehybridization buffer	5 x SSC, 1.0% (w/v) Blocking Reagent for nucleic acid hybridization, 0.1% N-lauroylsarcosine, 0.02% sodium dodecyl sulfate (SDS). When using RNA probes, add formamide to 50% and increase Blocking Reagent to 2 (w/v)*.
Standard hybridization buffer	DIG-labeled probe diluted in standard prehybridization buffer.
2 x wash solution	2 x SSC containing 0.1% SDS.
0.1 x wash solution	0.1 x SSC containing 0.1% SDS.

* Added from the Blocking Reagent stock solution (100 mM maleic acid, 150 mM NaCl, pH 7.5, containing 10% (w/v) Blocking Reagent for nucleic acid hybridization). See "Preparation of Additionally Required Solutions and Buffers" in Appendix C.

(Boehringer Mannheim Biochemica, 1993)

Appendix

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Neutralization solution 1	0.5 M Tris-HCl, pH 7.5; 3 M NaCl.
20 x SSC buffer	3 M NaCl, 300 mM sodium citrate; pH 7.0.
5 x SSC buffer	750 mM NaCl, 75 mM sodium citrate, pH 7.0.
Standard prehybridization buffer	5 x SSC, 1.0% (w/v) Blocking Reagent for nucleic acid hybridization, 0.1% N-lauroylsarcosine, 0.02% sodium dodecyl sulfate (SDS). When using RNA probes, add formamide to 50% and increase Blocking Reagent to 2 (w/v)*.
Standard hybridization buffer	DIG-labeled probe diluted in standard prehybridization buffer.
2 x wash solution	2 x SSC containing 0.1% SDS.
0.1 x wash solution	0.1 x SSC containing 0.1% SDS.
*Added from the Blocking Reagent stock solution (100 mM maleic acid, 150 mM NaCl, pH 7.5, containing 10% (w/v) Blocking Reagent for nucleic acid and hybridization). See Preparation of Additionally Required Solutions and Buffers in Appendix C.	

(Boehringer Mannheim Biochemica, 1993)

TO WHOM IT MAY CONCERN

I am writing this letter to confirm that Mr. Atit Koonsrisuk, a co-op student from the Suranaree University of Technology has successfully completed his four month work term in the Department of Mechanical Engineering under my supervision and the guidance of the

Two-Phase Flow Research Laboratory staff at the Technical University of Nova Scotia, Halifax, NS, Canada. Mr. Koonsrisuk's work term was between September to December, 1996, both inclusive. He was able to complete and report on every task and objective given to him to my satisfaction. I would be delighted to have him back either as a co-op student or as a practicing engineer with no reservation whatsoever.

Should you have any further questions please do not hesitate to call the undersigned.

Dated: January 10, 1997



Feridun Hamdullahpur, Ph.D, P. Eng.
Professor of Mechanical Engineering

Operation Report

“Introduction to Fluidization”

At:

Technical University of Nova Scotia
PO Box 1000
Halifax, Nova Scotia B3J 2X4
Canada

Submitted by:

Atit Koonsrisuk

Submitted to:

Dr.Feridun Hamdullahpur

12/10/96

5231 Morris St.

Halifax, Nova Scotia

B3J 1B7

December 10, 1996

Dr.F Hamdullahpur
Technical University of Nova Scotia, Faculty of Engineering
PO Box 1000
Halifax, Nova Scotia
B3J 2X4

Dear Dr.Hamdullahpur:

I came to be the research assistant at Technical University of Nova Scotia for a course of Co-operative Education II. The period is from September 20, 1996 to December 11, 1996. Please accept the attached report, "Introduction to Fluidization" in fulfillment of the operation report requirement for the course 401444 - Co-operative Education II.

I hope all the above is clear. And I thank you for all your help while I have been in Canada

Sincerely,

Atit Koonsrisuk

Atit Koonsrisuk

ACKNOWLEDGMENT

I am very grateful to Dr.Feridun Hamdullahpur, of Department of Mechanical Engineering of TUNS, and Anne Marie Coolen, of Co-operative Education office of TUNS, for their invaluable help in everything for me. I also wish to express my thanks to those people at TUNS for their kindness and warm support.

Many people helped to make this report what it is, in particular Levent Ersoy, the graduate student of Department of Mechanical Engineering of TUNS, whose comments helped in shaping the report, to edit the manuscript and eliminating many errors. Other graduate student of the same department who have been helpful is Murat Köksal, who gave me many explanation about Fluidization.

My girlfriend, Sorada Khaengkarn, encouraged me by e-mail to carry on with job.

Atit Koonsrisuk

December 1996

ABSTRACT

Suranaree University of Technology has determined that Co-operative Education be a part of the undergraduate curriculum. Students participate in full time work at the actual site, after they begin their major studies. There are two trimesters of co-operative education, each worth six credits. After the University has made the institutional linkage and technical co-operation agreement with the Canadian Universities of Technology Consortium, whose members are Ryerson Polytechnic University, University of Guelph, University of Waterloo and the Technical University of Nova Scotia, for joint implementation of a human resource development project under partial funding support from the Canadian International Development Agency (CIDA). In this term of Suranaree University of Technology, I had a chance to study in the Technical University of Nova Scotia (TUNS) to follow this project. Dr.Feridun Hamdullahpur, a professor in the Department of Mechanical Engineering at TUNS, hired me as a research assistant for his studies. After that I was assigned to work under the supervision of Levent Ersoy, a graduate student in mechanical engineering. My duties included:

- Assistance to the laboratory supervisor
- Mechanical design of experimental systems
- Utilization of commercial software for presentations
- Preparation of a technical report in the topic of "Introduction to Fluidization"

And sometimes in my free time, I would assist other graduate students in the same department to run their experiments.

I had chances to get many academic experiences and improve my skill in many fields, such as computer skill and English, from this work term. Furthermore I wrote my own report in the topic of "Introduction to Fluidization". The following report describes the parameters, phenomenon of fluidization and advantages and disadvantages of fluidized beds for industrial operations.

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Chapter 1

INTRODUCTION

1. Objectives of the report

First I expect that the undergraduate who have ever taken Thermodynamics and Fluid Mechanics should get the concept about fluidization. Next the researcher should be interested in using the definition that is presented. Furthermore I hope that this report could also be used as a beginning document for the student who is interested in Fluidization.

2. The details about The Technical University of Nova Scotia (TUNS)

Mailing Address:

Technical University of Nova Scotia
PO Box 1000
Halifax, Nova Scotia B3J 2X4
Canada
Phone: (902) 420-7500
FAX: (902) 420-7551

TUNS Historical Notes

The Technical University of Nova Scotia (TUNS) has a unique place in Canadian higher education. As an independent Canadian technical university, TUNS is dedicated to professional education and research in engineering, architecture, planning and computer science.

TUNS was founded as the Nova Scotia Technical College in 1907. Its mandate was to carry out research and offer degree programs in Engineering with the cooperation of universities and colleges in Nova Scotia and New Brunswick and to accept students with previous studies at the university level.

The College was established in a building on Spring Garden Road that is now occupied by the TUNS Faculty of Architecture. The original faculty of six taught courses in mining, metallurgical, civil, electrical and mechanical engineering. The first degrees were conferred in 1910 on nine students. Over the years, new departments of agricultural, chemical, food science, and industrial engineering were added.

In 1961, the TUNS School of Architecture was established. Today, degrees in environmental design, architecture and urban and rural planning are

offered through the Faculty of Architecture. More recently, the TUNS School of Computer Science was established. A cooperative degree is offered by the Faculty of Architecture, School of Computer Science and several engineering departments.

TUNS' commitment to graduate studies and research is worthy of special note. In the 1950s, graduate studies programs were developed, first at the Master's level, and then at the Ph.D. level.

To encourage research activities, TUNS supports, or is associated with, several research institutes and centers: Center for Water Resources Studies (CWRS); Canadian Institute of Fisheries Technology (CIFT); Center for Marine Vessel Design and Research (CMVDR); Vehicle Safety Research (VSRT); Minerals Engineering Center (MEC); Nova Scotia CAD/CAM Center; Nova Scotia Center for Environmentally Sustainable Economic Development (CESED); Advanced Materials Engineering Center (AMEC); Applied Microelectronics Institute (AMI); Atlantic Industrial Research Institute (AIRI). In addition, an active program in international development is pursued and there is a significant commitment to continuing education.

From a single building, TUNS has grown to a complex of buildings on the main campus at Spring Garden Road and Barrington Street. The facilities include a gymnasium and a student center, built with funds raised by alumni and corporate donations. Recently, additional nearby space has been leased to accommodate university programs.

3. The job position and the duties:

Name: Atit Koonsrisuk

Job position: research assistant

Job description:

- Assistance to the laboratory supervisor
- Mechanical design of experimental systems
- Utilization of commercial software for presentations
- Preparation of a technical report in the topic of "Introduction to Fluidization"

4. Job supervisor

In this section, there are the curriculum vitae of my job supervisors. The first is Dr.Feridun Hamdullahpur's, and the second is Levent Ersoy's.

CURRICULUM VITAE

Personal

Name: Hamdullahpur, Feridun
Birth Date: November 3, 1954
Marital Status: Married, Two children
Address: Department of Mechanical Engineering
Technical University of Nova Scotia
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B3J 2X4

Educational Background

May, 1985 Ph.D. in Chemical Engineering
Technical University of Nova Scotia
May, 1979 M.Sc. in Mechanical Engineering
Technical University of Istanbul
May, 1976 B.Sc. in Mechanical Engineering
Technical University of Istanbul

Academic Experience

1995-present Dean of Graduate Studies and Research
1993- 1995 Associate Dean of Graduate Studies and Research
1992-1993 Visiting Professor, Bosphorus University, Turkey
1995- present Professor Department of Mechanical
Engineering, TUNS
1990 - 1995 Associate Professor Department of Mechanical
Engineering, TUNS
1987 - 1990 Assistant Professor, Department of Mechanical
Engineering, TUNS

1985 - 1987	Assistant Professor, Faculty of Engineering, Center for Energy Studies, TUNS
1981 - 1985	Research Engineer, Centre for Energy Studies, TUNS; Lecturer, Chemical and Mechanical Engineering Departments, TUNS.
1977 - 1981	Research Engineer and Lecturer, Technical University of Istanbul

Thesis Work

Two Phase Flow Behaviour in the Freeboard of a Gas Fluidized Bed (Ph.D., 1985)

Loading of Solid Particles into the Main Transportation Line by a Jet-Pump (M.Sc., 1979)

Determination of the Suction Loss Coefficient for Moving Nozzles (B.Sc., 1976)

Industrial Experience

1980 - 1981 Consulting Engineer (Capacity analysis on pulp and paper industry), Istanbul Chamber of Industry

1979 - 1980 Consulting and Project Engineer (Hydraulic system design for food and beverage industry), Turkish Industrial Development Bank

1977 - 1978 Energy and Project Engineer (heating and ventilation systems), Gural Engineering and Consulting Company, Istanbul

PUBLICATION LIST

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6. CONFERENCE PRESENTATIONS

Katalambulla, H., P. R. Amyotte and F. Hamdullahpur, "The Effect of Distributor Parameters on Drying Rate of Coarse Particles in Fluidized Beds" International Conference on Agricultural Engineering, Uppsala, Sweden, Poster No. 9203-108, June 1992

Ergudenler, A., A. Ghaly, F. Hamdullahpur, and A. M. Al Taweel, "Fluidized Bed Gasification of Cereal Straw" Paper presented at 73rd Canadian Chemical Conference, Halifax, N.S., 1990.

BasiratTabrizi, H., A.M. Al Taweel, and F. Hamdullahpur, "Mathematical Modelling of Gas solid Distribution in a Cyclone Separator", Paper presented at 73rd Canadian Chemical Conference, Halifax, N.S., 1990

Al Taweel, A.M., P.R. Amyotte, P. Basu, A. Ghaly, F. Hamdullahpur, G. D. M. MacKay, and J.Militzer, "An Overview of Fluidization and Fluidized Bed Combustion Research at the Technical University of Nova Scotia", Presented at 73rd Canadian Chemical Conference, Halifax, N.S., 1990

Al Taweel, A.M., J.H.Kan, F.Hamdullahpur, and J.Militzer, "Monitoring the Motion of Aggregates in the Freeboard of a Bubbling Fluidized Bed", Paper presented at 73rd Canadian Chemical Conference, Halifax, N.S., 1990

Zayed, R.S., F. Hamdullahpur, and A.M. Al Taweel, "Effect of Secondary Swirling Air on the Behaviour of a Freeboard of a Fluidized Bed", Paper presented at 73rd Canadian Chemical Conference, Halifax, N.S., 1990

Katalambulla, H., P.R.Amyotte, and F.Hamdullahpur, "Fluidized Bed Drying of Coarse Uniform Size Particles", Paper presented at 73rd Canadian Chemical Conference, Halifax, N.S., 1990

Hamdullahpur, F. "Fluidization and FBC Research Activities at TUNS. Results of Solid Phase Measurements in the Freeboard with Secondary Air Injection" The 3rd National Canadian Institute of Fluidized Bed Technology Technical Workshop, Fredericton, N.B., 1989 (Invited Speaker)

Busaiffi, M.A., A.M. Al Taweel, and F. Hamdullahpur, "Pipeline Transportation of Oil Emulsions" Presented at the 38th Canadian Chemical Engineering Conference, Edmonton, October 1988.

Al Taweel, A.M., J. Militzer, J.M. Kan, F. Hamdullahpur and M. Fayed, "Sedimentation of Flocculated Particles", CSChE 1987 Centennial Meeting, Montreal, PQ, May 1987.

Zhou, Z.Q., G.S. Trivett and F. Hamdullahpur, "Further Improved Operation of an Atmospheric Pressure Fluidized Bed using Coal/Water Mixtures", Proceedings of the Sixth International Workshop on Coal-Liquid and Alternate Fuels Technology, Halifax, N.S., 1986.

Hamdullahpur, F., G.S. Trivett, R.B. Boak and J.C. Campbell, "Performance Tests of a Light Industrial (150 kW) AFBC Fuelled with Coal-Water Slurry", Proceedings of the Sixth International Workshop on Coal-Liquid and Alternate Fuels Technology, Halifax, N.S., 1986.

7. COURSE NOTES

Hamdullahpur, F. "Energy Conversion Systems" Technical University of Nova Scotia (TUNS)

8. SUPERVISED THESES (COMPLETED)

M. Gagne, "Turbulence modeling of swirling gas-particle flow in a sudden expansion geometry" TUNS, 1996, (MASC)

A. Ergudenler, "Fluidized Bed Gasification of Cereal Straw" TUNS, 1992, (PhD) (Dr. A Ghaly, Co-supervisor)

D. G. DiMattia, "Onset of Slugging in Fluidized Bed Drying of Large Particles" TUNS, 1992, (MASC)

A. S. Fung, "Particle Dynamics in the freeboard of a Fluidized Bed" TUNS, 1991, (MASC)

W. McGregor, "A Technoeconomic Evaluation of Fluidized Bed Furnaces for Space and Hot Water Heating" TUNS, 1991 (MASC), (Dr. I. Ugursal, Co-supervisor)

A. El-Aghili, "Heat Transfer in Fluidized Bed Furnaces" TUNS, 1991 (MASC)

H. Basirattabrizi, "Modelling of Two-phase Flow in a High Temperature Cyclone Separator" TUNS , 1990, (PhD)

R. S. Zayed, " Particle Motion in the Freeboard of Swirling Fluidized Bed" TUNS, August 1990, (PhD)

H. Katalambulla, " Fluidized Bed Drying of Coarse Uniform Sized Particles" TUNS, April 1989, (MAsc) (Technical Supervisor)

M. Busaiffi, " Pipeline Transportation of Oil Emulsions " TUNS, April 1989, (MAsc) (Dr. A. M. Al Taweel, Co-supervisor)

Participated in the organization of the following conferences and workshops:

Session Chairman, 9th International Conference on Fluidized Bed Combustion, May, 1987, Boston, MA.

Assistance Conference Chairman - The First International Conference on Circulating Fluidized Bed, Halifax, N.S., November 16, 1985.

Member, Editorial Board - An International Workshop on the Design and Operation of Atmospheric Pressure Fluidized Bed Boiler Combustor, Halifax, N.S., June 23, 1983.

Areas of Interest

- *Applied Fluid Mechanics and Heat Transfer*
- *Energy Conversion*
- *Combustion, Incineration*
- *Fluidization and Fluidized Bed Combustion*
- *Two-Phase Flow*
- *Non-Intrusive (LDV) Flow Diagnostics*

Professional Memberships

- *Association of Professional Engineers of Nova Scotia*
- *American Society of Mechanical Engineers*
- *Canadian Society of Chemical Engineering*
- *Chemical Institute of Canada*
- *Canadian Combustion Institute*
- *American Flame Research Foundation*

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Educational

January 1994 - Present *TUNS (Technical University of Nova Scotia), Halifax, Canada*
Ph. D candidate in Mechanical Engineering
Subject: Experimental And Computational Two Phase Flow
Expected graduation date : September-1997

1990-93 *MIDDLE EAST TECHNICAL UNIVERSITY (METU), Ankara, Turkiye*
MS in Engineering Sciences
Solid Mechanics: Fracture mechanics. Advanced stress analysis.
Thesis on "Stress Analysis of a Long Cylinder Under Different Shaped Indenters"

1984-89 *MIDDLE EAST TECHNICAL UNIVERSITY (METU), Ankara, Turkiye*
BS in Mechanical Engineering
Concentration on Thermodynamics, HVAC, Combustion, Gas Turbines and IC Engines

Work Experience

January 1994-Present *TECHNICAL UNIVERSITY OF NOVA SCOTIA, Halifax, NS*
Teaching and Research assistant in the department of "Mechanical Engineering"
-Tutorials and assignment marking for Thermodynamics I and Thermodynamics II courses.
-Tutorials and assignment marking for Heating Ventilating and Air Conditioning Course.
-Tutorials and assignment marking for Steam Power Plant course.
-Lab assistant for Heating Ventilating and Air Conditioning, Refrigeration and Compressors Labs
-Lab assistant for Non Intrusive Flow Measurements Lab (Maintenance and upgrading of Laser Doppler Velocimeter equipment)

- Research papers on "Two Phase Flow", "Fluidization", "Fluidized bed Combustion"
- Experimental works on "Design and Production of a Laboratory scale Circulating Fluidized Bed"
- Measurement of Particle Velocity and Velocity Fluctuations in CFB's with an optical probe",
- Measurement of Pressure and Pressure Fluctuations in Gas- Solid Two Phase Flow"
- Fluidized Bed Drying".

April 1991- January 1994,

ASELSAN MILITARY ELECTRONICS Inc., Ankara, Turkey

Production Engineer responsible from Electromechanic Assembly and Cable Harness lines.

- Worked for the design of fully automated production lines. Designed necessary jigs, fixtures and apparatus for efficient capacity usage and productivity purposes.
- Implemented ManMan Production software to shop floor for MRP, MPS and capacity planning purposes.
- Familiar with the assembly of electromechanical parts. Hand soldering, wave soldering, insertion machines, printed circuit boards, flexible cables, ESD control.
- Attended a series of seminars on 'Total Quality Management', and implemented the principles of TQM to the production personnel. Organized quality circles among the 70 technicians and lead the quality circle for 'Material Handling and Plant Layout' committee.
- Designed an efficient test set-up for the 'water tightness' requirements according to MIL standards.
- Prepared the departments successfully to get the ISO 9000 Certificate,
- Advised the mechanical R&D team in designing 9600 Family frequency hopping military radio receiver and transmitters for productivity.
- Timing and rooting control.
- Supervised the electromechanical production lines of : Philips 4600 family radio receiver and transmitter, FT 6200- family field telephones, VT 7220 Proximity Fuse (Mechanical; production and foaming, Philips licensed), CX - series power cables, and cable harnesses for the above devices. Antenna cables.

June 1987-Sept. 1987,

KUTLUTAS Inc., Ankara, Turkiye

Student Engineer. Worked in the production lines of mixer trailers. Assisted the machine operators for every station. Designed a more efficient machine layout for the production lines

Worked for the accounting office for one week

June 1986 - Sept. 1986,

EFE TARIM Inc., Izmir, Turkiye

Student Engineer. Worked in the production lines of agricultural goods: Trailers and Harvesting machines. Assisted the operators in the production of hydraulic pistons and cylinders.

Performed the quality endurance tests on the hydraulic garbage collecting trucks.

1983-Present

PRIVATE

Professor

- Tutoring, English and Mathematics and Physics to the secondary and high school students

1986-1991 Ankara Kolejliler Spor Klubu, Ankara, Turkiye.

Semi - Pro. Athlete Played basketball in the Turkish National Basketball League.

1991-1993 Mulkiye Spor Klubu, Ankara, Turkiye.

Semi - Pro. Athlete. Basketball Team captain.

Academic and Social Achievements

- Attended an international seminar for one week on 'Material Handling and Plant Layout'. Middle East Technical University, Ankara, Turkiye.
- Attended Production Machines and Equipment Fair' 1993, Hannover, Germany
- Proud to be a starting lineup member of METU'88, Turkish Universities Basketball League Champion Team.
- Played for Nova Scotia All Star Basketball Team - Hall of Fame - December'94, Halifax
- All Star -Atlantic Collages Athletic Association '1993-94, 94-95
- Most Valuable Player - TUNS Varsity Basketball Team '1994-95
- Striker, TUNS Varsity Soccer Team '1995-96
- Honors during high school and university years
- Member of Mechanical Engineering Association, Ankara, Turkiye
- Mechanical Engineering Department Representative in Graduate Student Society 1994 - Present
- Secretary, TUNS International Student Organization, 1995- Present
- Traveled All Around Europe, Eastern United States and Cuba
- Reid Schollar-1995.

Skills & Abilities

- Languages Turkish, English and have been taking French
- Programs in Turbo Pascal, FORTRAN, C, C++, Labview, Basic, Visual Basic
- Operating systems: UNIX, Makintosh DOS and Windows.
- Computational Fluid Dynamics and Stress analysis: FLUENT, PHOENICS, ALGOR.
- Drafting: AUTOCAD,
- Data acquisition and control: LABVIEW.
- Numerous DOS/Windows software for Mathematical and statistical analysis packages, spreadsheets, word processors, etc.
- Manufacturing Management Software MAN-MAN
- Quick to learn any computing software
- Capable of employing advanced mathematical and physical modeling.
- Performant in individual and team work
- Able to efficiently communicate at all levels of an organization
- Familiar with international environments
- Landed Immigrant of Canada
- Practical, self initiative, dynamic
- References on request-

Chapter 2

Introduction to Fluidization

Fluidization

Fluidization is a process in which the particles are lifted by the drag of the fluid and the mass of particles looked like a boiling liquid. On the other hand, it is the phenomenon that the solid particles behave as a fluid with a density as that of the solids and fluid combined.

If the fluid passed upwards through the bed, the pressure drop will be directly proportional to the rate of flow, when the frictional drag on the particles becomes equal to their apparent weight (actual weight less buoyancy), the particles become rearranged so that they offer less resistance to the flow of fluid and the bed starts to expand. This process continues as the velocity is increased, with the total frictional force remaining equal to the weight of the particles, until the bed has assumed the loosest stable form of packing. If the velocity is then increased still further, the individual particles separate from one another and become freely supported in the fluid and the bed is said to be *fluidized*. Further increase in the velocity causes the particles to separate still further from one another, and the pressure difference remains approximately equal to the weight per unit area of the bed.

A fluidization vessel usually consists of two zones, a *dense bubbling phase* having a more or less distinct upper surface separation it from a *lean or dispersed phase*. The section of the vessel between the surface of the dense phase and the exiting gas stream from the vessel is called the *freeboard*, and its height is called the *freeboard height* H . Some of the particles in the freeboard zone are carried a significant distance above the bed surface and are elutriated, whereas others simply fall back to the surface. Eventually the rate of entrainment appears nearly constant, the freeboard height at this point is referred to as the *transport disengaging height* TDH.

The use of the fluidized solids technique was developed very largely by the petroleum and chemical industries, for processes where the very high heat transfer coefficients and the high degree of uniformity of temperature within the bed enabled the development of processes which would otherwise be impracticable. Fluidized solids are now used quite extensively in many industries where it is desirable to bring about intimate contact between small solid particles and a gas streams. In many cases it is possible to produce the same degree of contact between the two phases with a very much lower pressure drop over the system. Drying of finely divided solids is now carried out in a fluidized system, and some carbonization and gasification processes are now in

operation. Fluidized beds are employed in gas purification work, in the removal of suspended dusts and mists from gases, in lime burning and in the manufacture of phthalic anhydride.

The Phenomenon of Fluidization

If a fluid is passed upward through a bed of fine particles, as shown in Fig. 1(a), at a low flow rate, the fluid merely percolates through the void spaces between stationary particles. This is a *fixed bed*. With an increase in flow rate, particles move apart and a few vibrate and move in restricted regions. This is the *expanded bed*.

At a still higher velocity, a point is reached where all the particles are just suspended by the upward-flowing gas or liquid. At this point the frictional force between particle and fluid just counterbalances the weight of the particles, the vertical component of the compressive force between adjacent particles disappears, and the pressure drop through any section of the bed about equals the weight of fluid and particles in that section. The bed is considered to be just fluidized and is referred to as an *incipiently fluidized bed* or a bed at *minimum fluidization*; see Fig. 1(b).

In liquid-solid systems, an increase in flow rate above minimum fluidization usually results in a smooth, progressive expansion of the bed. Gross flow instabilities are damped and remain small, and heterogeneity, or large-scale voids of liquid, are not observed under normal conditions. A bed such as this is called a *particulately fluidized bed*, a *homogeneously fluidized bed*, or a *smoothly fluidized bed*; see Fig. 1(c). In gas-solid systems, such beds can be observed only under special conditions of fine light particles with dense gas at high pressure.

Generally, gas-solid systems behave quite differently. With an increase in flow rate beyond minimum fluidization, large instabilities with bubbling and channeling of gas are observed. At higher flow rates, agitation becomes more violent and the movement of solids become more vigorous. In addition, the bed does not expand much beyond its volume at minimum fluidization. Such a bed is called an *aggregative fluidized bed*, a *heterogeneous fluidized bed*, or a *bubbling fluidized bed*; see Fig. 1(d). In a few rare cases, liquid-solid systems also behave as bubbling beds. This occurs only with very dense solids fluidized by low-density liquids.

Both gas and liquid fluidized beds are considered to be dense-phase fluidized beds as long as there is a fairly clearly defined upper limit to the bed.

In gas-solid systems, gas bubbles coalesce and grow as they rise, and in a deep enough bed of small diameter they may eventually become large enough to spread across the vessel. In the case of fine particles, they glow smoothly down by the wall around the rising void of gas. This is called *slugging*, with *axial slugs*, as shown in Fig. 1(e). For coarse particles, the portion of the bed above the bubble is pushed upward, as by a piston. Particles rain down from the slug, which finally disintegrates.

At about this time another slug forms, and this unstable oscillatory motion is repeated. This is called a *flat slug*; see Fig. 1(f). Slugging is especially serious in long, narrow fluidized beds.

When fine particles are fluidized at a sufficiently high gas flow rate where the terminal velocity of the solids is exceeded, the upper surface of the bed disappears, entrainment becomes appreciable, and, instead of bubbles, one observes a turbulent motion of solid clusters and voids of gas of various sizes and shapes. This is the *turbulent fluidized bed*, shown in Fig. 1(g). With a further increase in gas velocity, solids are carried out of the bed with the gas. In this state we have a *disperse-, dilute-, or lean-phase fluidized bed with pneumatic transport of solids*; see Fig. 1(h).

In both turbulent and lean-phase fluidization, large amounts of particles are entrained, precluding steady state operations. For steady state operation in these contacting modes, entrained particles have to be collected by cyclones and returned to the beds. In turbulent fluidized beds, inner cyclones can deal with the moderate rate of entrainment, as shown in Fig. 2(a), and this system is sometimes called a *fluid bed*. On the other hand, the rate of entrainment is far larger in lean-phase fluidized beds, which usually necessitates the use of big cyclone collectors outside the bed, as shown in Fig. 2(b). This system is called the *fast fluidized bed*.

In fluid beds and fast fluidized beds, recirculation of solids through the dipleg or other solid trapping device is crucial for smooth operations. These beds are called *circulating fluidized beds*.

The *spouted bed*, sketched in Fig. 3, represents a somewhat related contacting mode wherein comparatively coarse uniformly sized solids are contacted by gas. In this operation, a high-velocity spout of gas punches through the bed of solids, thereby transporting particles to the top of gas punches through the bed of solids, thereby transporting particles to the top of the bed. The rest of the solids move downward slowly around the spout and through gently upward-percolating gas. Behavior somewhere between bubbling and spouting is also seen, and this may be called *spouted fluidized bed* behavior.

Compared to other methods of gas-solid contacting, fluidized bed have some rather unusual and useful properties. This is not the case with liquid-solids fluidized beds. Thus, most of the important industrial applications of fluidization to date are with gas-solid systems.

Identification of fluidization parameters

1. Superficial gas velocity, u_c

Superficial gas velocity is the gas velocity in the containing vessel that if there were not any solid particles in it. It can be calculated from the ratio of the volume flow rate of the fluid to the cross section area of the containing vessel.

$$u_c = \frac{\dot{V}}{A_c}$$

u_c is the superficial gas velocity.

$\dot{V}$ is the volume flow rate.

A_c is the cross section area of the column.

2. Interstitial gas velocity, u_i

Interstitial gas velocity is the gas velocity in the containing vessel that there are the solid particles in it. It can be calculated from the ratio of the volume flow rate of the fluid to the outcome of the cross section area of the containing vessel minus the area that possessed by the solids particles in the same plane.

$$u_i = \frac{\dot{V}}{A_c - A_{solids}}$$

u_i is the interstitial gas velocity.

A_{solids} is the area that possessed by the solids particles in the same plane with the cross section area of interest.

The interstitial gas velocity is defined as the average velocity of the gas in containing vessel that there are the solid particles in it. However when we want to define any velocity, such as, minimum fluidization velocity or minimum bubbling velocity, we will define based on the superficial gas velocity. Since interstitial gas velocity is not constant throughout the bed, its value changes with the cross sectional solid concentration.

3. Minimum fluidization velocity, u_{mf}

It is the superficial gas velocity at which the weight of the particles in the bed is counterbalanced by the drag force on them. As the upward velocity of flow of fluid through a packed bed(see fixed bed) of uniform spheres is increased, the point of incipient fluidization is reached when the particles are just freely supported in the fluid.

Most of the bubbling beds are designed by using the minimum fluidization velocity as a major parameter, it is desirable to standardize a method of determination so that the characteristics of different systems can be compared. This is most conveniently done by using the graph of frictional pressure drop against velocity. If separate lines are drawn through the points for the fixed(flow decreasing) and fluidized regions, and the points in the intermediate region are ignored the point of

intersection of these two lines will give a reproducible value for the minimum fluidization velocity. The whole of the bed is not fluidized until all the particles are fully supported in the fluid and the pressure drop becomes exactly equal to the buoyant weight per unit area Δp_{eq} . The minimum velocity at which this occurs will be called the "full supporting velocity", u_{fs} . This is a difficult quantity to determine accurately because the pressure drop reaches its limiting value gradually. Furthermore, the value of u_{fs} may well be influenced by the initial packing of the solids and the bed support, and may not therefore have a consistent value for any particular system. For the experimental results, u_{fs} is more than 50% greater than u_{mf} . The whole problem of measurement can be complicated too, especially in beds of small diameter, by frictional effects between the circulating solids and the walls of the container.

In the absence of the facility to carry out an experimental determination, it is useful to be able to calculate approximately the value of the minimum fluidization velocity. This may be done by using an expression for the relation between pressure drop and superficial gas velocity for a fixed bed and putting the pressure drop equal to the buoyant weight of the particles. This does, of course, necessitate a knowledge of the voidage of the bed at the minimum fluidizing velocity (e_{mf}). This will depend on the shape and size range of the particles, but a value of about 0.4 would be appropriate for approximately spherical particles. Various attempts have been made to relate the value of e_{mf} to a shape factor for the particle, but these have not been entirely satisfactory.

The corresponding minimum fluidization velocity (u_{mf}) is obtained by the following procedure [Coulson & Richardson, 1991].

From

$$G_a = \left(150 \times \frac{1 - e_{mf}}{e_{mf}^3} \times Re'_{mf} \right) + \left(\frac{1.75}{e_{mf}^3} \times Re'^2_{mf} \right)$$

By definition:

$$u_{mf} = \frac{\mu}{d \cdot \rho} Re'_{mf} \quad \text{-----} \textcircled{1}$$

$$G_a = \frac{d^3 \cdot \rho \cdot (\rho_s - \rho) \cdot g}{\mu^2}$$

For a typical value of $e_{mf} = 0.4$:

$$(Re'_{mf})_{e_{mf}=0.4} = 25.7 \times \left\{ \sqrt{1 + 5.53 \times 10^{-5} Ga} - 1 \right\} \quad \text{-----} \textcircled{2}$$

And substitute the value of Re'_{mf} from equation $\textcircled{2}$ into equation $\textcircled{1}$. Then the corresponding value is obtained. (see the example that the minimum fluidization velocity is calculated in Appendix A.)

4. Minimum bubbling velocity, u_{mb}

In gas solid fluidized beds, only a bed limited expansion takes place as the gas velocity is increased from a minimum fluidization velocity. At higher gas flow rates much of gas passes through the bed in the form of bubbles and makes a relatively small contribution to the expansion of the bed. The upper limit of gas velocity for particulate expansion is termed as the minimum bubbling velocity, u_{mb} . There are a lot of difficulties; because its value may depend on the nature of the distributor, on the presence of even tiny obstructions in the bed, and even on the immediate history of the bed. The ratio u_{mb}/u_{mf} gives a measure of the degree of expansion of the bed. The ratio might also be considered as a measure of the sizes of the bed particles. It usually has a high value for fine light particles and a low value for large dense particles.

5. Terminal velocity

If a particle of mass m and weight mg , initially at rest, is allowed to fall under the action of gravity force, at some later time, the particle will accelerate to a definite velocity. During its downward flight the particle is subjected to the resistance of the surrounding fluid medium. This resistance increases as the particle velocity increases up to the point in time when accelerating and resisting forces are equivalent. From this point, the particle continues to fall at a constant maximum velocity, known as the terminal velocity.

6. Entrainment

In general, entrainment refers to the removal of solids from the bed by fluidizing gas in both single component and multicomponent systems. For a freeboard height less than TDH, the size distribution of solid in the freeboard changes with position, and entrainment decreases as the freeboard height approaches the TDH. When the gas stream exits above the TDH, then both the size distribution and entrainment rate become constant and are given by the *saturation carrying capacity* of the gas stream under pneumatic transport conditions. This term also applies to the entrainment above the TDH for a single size of solid.

7. Elutriation

Elutriation refers to the selective removal of fines by entrainment from a bed consisting of a mixture of particles sizes. The rate of elutriation of solids of size d_p from a mixture is characterized by the net upward flux of this size of solid,

$$\left(\begin{array}{l} \text{rate of removal of solids} \\ \text{of size } d_p \text{ per area of bed} \\ \text{surface, gm / cm}^2 \cdot \text{sec} \end{array} \right) = \chi^* \left(\begin{array}{l} \text{fraction of bed} \\ \text{consisting of} \\ \text{size } d_p \end{array} \right)$$

If $W(d_p)$ is the weight of solid of size d_p , A_t is the cross-sectional area of bed and W is the weight of all solids in the bed, then the equation become

$$-\frac{1}{A_t} \frac{dW(d_p)}{dt} = \chi^* \left(\frac{W(d_p)}{W} \right)$$

where χ^* ($\text{gm/cm}^2 \cdot \text{sec}$) is an elutriation (rate) constant. In a mixture χ^* varies with size of solids; a large value for χ^* corresponds to a rapid removal rate, and $\chi^* = 0$ means that particular size of solid is not removed at all by entrainment.

The elutriation rate is also defined by

$$\left(\begin{array}{c} \text{rate of removal} \\ \text{of solids of size } d_p \end{array} \right) = \chi \left(\begin{array}{c} \text{weight of that size} \\ \text{solid in the bed} \end{array} \right)$$

or

$$-\frac{dW(d_p)}{dt} = \chi W(d_p)$$

In comparing definitions we see that

$$\chi^* = \chi \frac{W}{A_t}$$

Although χ (sec^{-1}) is also called an elutriation constant, one should note that it varies proportionately with bed section and inversely with bed height. Also, in batch or unsteady-state experimentation χ should change during a run as material is elutriated from the bed (W changes). The constant χ^* is unaffected by these changes as long as the quality of fluidization remains the same.

8. Voidage

Depending on the shape of particles and packing characteristics, a certain volume of space in between the particles remains unoccupied such space is called a void volume and is specified as voidage or porosity, defined as

$$\text{voidage} = \text{porosity} = \frac{\text{void volume}}{\text{Total volume}}$$

9. Emulsion phase

Emulsion phase is the region of high solids density in a fluid bed, whereas, those of low solids density are referred to as gas pockets or voids.

When we increase the gas flow through the solids bed, this act can cause the gas to flow in the form of *bubbles*. The section of the bed outside the bubbles is the emulsion phase.

10. Fixed Bed

Consider a column that is filled with loose solids. If a fluid is passed upward through the bed of solids at a relatively low velocity, then the fluid will merely percolate through the void spaces between solid particles. The solid particles in this case will remain undisturbed. This system is referred to as a *fixed or packed bed*. In *moving packed beds*, the solids move with respect to the wall of the column. In either case the particles do not move relative to each other. As the gas flows through the solids, it exerts a drag force on the particles, causing a pressure drop across the bed. the pressure drop through unit height of a fixed bed of uniformly sized particles, $\Delta P/L$, is correlated as (Basu & Fraser, 1991)

$$\frac{\Delta P}{L} = 150 \frac{(1-\varepsilon)^2}{\varepsilon^3} \cdot \frac{\mu u_c}{(\phi d_s)^2} + 1.75 \frac{(1-\varepsilon)}{\varepsilon^3} \cdot \frac{\rho_g u_c^2}{\phi d_s}$$

where u_c is the superficial gas velocity
 d_s is the diameter of bed particles
 ϕ is the sphericity of bed particles ¹
 μ is the viscosity of fluid
 ρ_g is the gas density

¹ Sphericity describes the departure of the particle from a spherical shape, for example, a spherical particle has a sphericity of 1.0

$$\text{Sphericity } (\phi) = \frac{\text{Surface area of a sphere with the volume same as the particle}}{\text{Actual surface area of the particle}} = \frac{\pi d_v^2}{S}$$

d_v (Volume diameter) is the diameter of a sphere that has the same volume as the particle

$$d_v = \left(\frac{6}{\pi} \times \text{volume of particle} \right)^{\left(\frac{1}{3} \right)} = \left(\frac{6V}{\pi} \right)^{\left(\frac{1}{3} \right)}$$

11. Bubbling fluidized bed

If the gas flow rate through the fixed bed is increased, the pressure drop continues to rise, until the superficial gas velocity reaches the minimum fluidization velocity. At this stage the particles feel "weightless", and the fixed bed transforms into an incipiently fluidized bed. In this regime the body of solids behaves like a liquid.

12. Turbulent bed

When the velocity of gas through a bubbling fluidized bed is increased above the minimum bubbling velocity, the bed expands. A continued increase in the velocity may eventually show a change in the pattern in the bed expansion. This may be due to an increase in the bubble fraction, an expansion of the emulsion phase, and thinning of the emulsion walls separating the bubbles. At this stage the bubble phase loses its identity due to rapid coalescence and breakup. This results in a violently active and highly expanded bed, and a change in the pattern of bed expansion. Particles are thrown into the freeboard (freeboard is the zone of the vessel, between the surface of the dense bed and the top of the bed where the gas stream exits) above the bed. Such beds are referred as the turbulent beds.

13. Fast Bed

The fast bed may be defined as a high velocity gas-solid suspension where particles, elutriated by the fluidizing gas above the terminal velocity of single particles are recovered and returned to the base of the furnace at a rate sufficiently high as to cause a degree of solid refluxing that will ensure a minimum level of temperature uniformity in the furnace.

It was described as a regime lying between the turbulent fluidized bed and the pneumatic transport (see pneumatic transport). In a typical fast fluidized bed, one observes a non-uniform suspension of slender particle agglomerates moving up and down in a very dilute up-flowing gas-solid continuum.

14. Pneumatic transport

If the gas is flowing upwards through a vertical column to which solid is fed at a given rate. The suspension is initially in pneumatic transport.

The definition of pneumatic transport is similar with fast bed. But in pneumatic transport, solid particles merely flow upwards through the column. In fast bed, there are the solid particles flow upward and downward in the column.

Transition from pneumatic transport to fast fluidization may occur when the rate of solid circulation is increased while keeping the gas velocity fixed above a certain velocity.

15. Circulating fluidized bed

As the velocity of gas flow through a fluidized bed of fine powder was raised, the breakdown of the slugging regime into a state of continuous coalescence, is observed. The bed has the appearance of turbulence and if solid is introduced into the bottom of the fluidized bed and withdrawn from the top, we have the recirculating fluidized bed operating in the turbulent regime. This recirculating fluidized bed has been termed "circulating fluidized bed".

16. Distributor plate

Distributor plate is a perforated grid which a bed of particles sit on. Its diameter is smaller than the diameter of particles; but the gas from the blower can pass through it.

Types of Distributors

Distributor plates can be broadly classified in to three groups:

1. *Porous* and straight-hole orifice type: it generally uses vertical orifices through a grid plate or sintered plates.
2. *Nozzle* type or bubble caps: air enters into nozzles, which distribute air into the bed through horizontal or vertically downward holes.
3. *Sparge tube* type: air-carrying tubes with holes are introduced directly into the fluidized bed; does not require a grid plate or a *plenum box* (the section between the air inlet jet and the distributor plate) below it.

Typical designs of above type of distributors are shown in Figure 4. In addition to these, there are a few novel designs used by some companies with considerable success. For example, the bent-tube nozzle used by Pyropower Corporation (Figure 4), prevents back-flow of solids and is easy to clean up.

Desired Properties

The primary function of the distributor grate is to distribute the fluidizing air uniformly over the cross section of the bed. The formation of bubbles (in the external heat exchanger or seal pot) or the random mixing in the bed often changes local pressure above the gas distributor. This causes the flow through individual orifices to change. More air flows through the orifices that are subjected to lower downstream pressures. Thus channels or flow paths of low voidage are established above these orifices, which further increases the flow through these orifices, while depriving other orifices. This leads to a nonuniform flow. For example, solids flowing along the wall create a dense region near the wall. As a result, the flow through orifices near the wall experiences a high downstream pressure and, therefor, a smaller amount of air flow through it.

The desired properties of the distributor may be listed as follows:

1. Uniform and stable fluidization over the entire range of operation
2. Minimum attrition of bed materials
3. Minimum erosion of bed internals or heat exchanger tubes.
4. Minimum back-flow of solids into the plenum chamber
5. Minimum amount of dead zones on the distributor
6. Minimum plugging over extended periods of operation.

17. Cyclones

A cyclone is a device which separates solid particulate from a relatively dry gas stream. Hydroclones are essentially the same device, which effect solids separations from liquids. Both devices are the simple and economical separators. Their operation is identical, whereby both inertial and gravitational forces are capitalized upon. Their primary advantages are high collection efficiency in certain applications, adaptability and economy in power. The main disadvantage lies in their limitation in achieving high collection efficiencies for small particles. In general, cyclones are not capable of high efficiencies when handling gas streams containing large concentrations of particulates less than 10 μm in size.

Cyclones generally are efficient handling devices for a wide range of particulate sizes. They are capable of collecting particles ranging in size from 10 to above 2,000 μm , with inlet loading from less than one grain per scfm to greater than 100 grains per scfm. There are many design variations of the basic cyclone configuration. Because of its simplicity and lack of moving parts, a wide variety of construction materials can be used to cover relatively high operating temperatures of up to 2000°F.

Cyclones are employed in the following general applications:

1. Collection of coarse dust particles
2. Handling high solid concentration gas streams between reactors such as Flexicokers (concentration typically above 3 grains/sfc)
3. For classifying particulate sizes
4. In operations where extremely high collection efficiency is not critical
5. As precleaning devices in line with higher efficiency collectors for fine particles

18. L-valve

L-valve is one popular type of nonmechanical valve. It's a controllable valve. Nonmechanical valves are devices that allow the flow of solids between the return leg (standpipe) and the furnace without any external mechanical force. Solids in nonmechanical valve are moved by air. This air is added at a short height above the exit of the valve. The bulk of the aeration air generally flow in the desired direction of solid flow. This is

governed by the pressure balance around the valve. The L-valve consists of a right-angled, bent (L-shaped) pipe connecting the two vessels between which the solids are to be transferred. In a CFB boiler the vertical leg of the L-valve is connected to the solid hopper of the cyclone(or any gas-solid separator). The horizontal arm of the L-valve is connected to the CFB furnace. The L-valve would usually have a moving packed bed in the vertical leg and a less dense bed in the furnace into which the solids is fed. A small amount of air is injected into the vertical leg of the L-valve at a short distance above the axis of its horizontal arm. These air (or gas) moves solids from the dense vertical leg to the relatively dilute bed. The aeration air moves downwards through the particles and then through the constricting bend of the L-valve. Solid flow commences only beyond a certain minimum value of air rate.

19. Bed material

In fluidized bed coal combustion processes, coal generally constitutes around 1-3% by weight of the total solids in the bed. The remaining solids, known as bed materials, are coal ash and reacting or spent sorbents. The bed material includes

1. Sand or gravel (low ash fuels, such as wood-chips).
2. Fresh or spent limestone(boilers burning high-sulfur coal and requiring control of sulfur emission).
3. Ash from coal(boilers firing high or medium ash coal requiring no sulfur retention).

Particle Classification

In the light of fluidization experience, Geldart (1973) classified solids broadly under four groups, **A**, **B**, **C** and **D**. This classification is important in understanding the fluidization behavior of solids particles, because under similar operating conditions particles of deferent groups may behave entirely differently.

Group A

These particles (density of solids, $\rho = 2500 \text{ kg/m}^3$) are typically in the range of 30-100 μm . These particles fluidized well, but expand considerably after crossing the minimum fluidization velocity and before bubbles start appearing. Many circulating fluidized bed systems use Group A particles.

Group B

These particles are normally in the range of 100-500 μm (if $\rho = 2500 \text{ kg/m}^3$) size. They fluidize well. Bubbles appear as soon as the minimum fluidization is exceeded. The majority of the CFB boilers use this group of particles.

Group C

These particles are very fine and are typically smaller than $30\text{ }\mu\text{m}$ ($\rho = 2500\text{ kg/m}^3$). The interparticle forces are comparable to the gravitational force on these particles. These particles are very difficult to fluidize. An attempt at fluidization often results in channeling. Special techniques are required to fluidize these particles.

Group D

These are the coarsest of all particles ($> 500\text{ }\mu\text{m}$) (for $\rho = 2500\text{ kg/m}^3$). They require a much higher velocity to fluidize these solids. Spouted beds (see The Phenomenon of Fluidization) generally operate on this size of solids.

A comparison of properties of particles of different groups is given in Table 2

20. Riser

Solids can be transported up or down an inclined or vertical pipe. Such a pipe, with solids traveling downwards cocurrently or countercurrently to a fluid, is referred to as a standpipe. A pipe in which solids are lifted upwards by a fluid is known as a riser. Standpipes and riser together often form part of a solid circulatory loop. The solid flow rate may be controlled by means of a slide valve affixed to the bottom end of the standpipe or by other means.

21. Downer

Downer is the crucial element in a pneumatic circulation system for solids return, its function being to transfer solids from an upper zone or vessel at low pressure to a lower zone of higher pressure while providing a gas seal between vessels. In this operations, gas tries to flow up the pipe to the low-pressure region but is hindered by the downflowing solids [Flu. Eng. P.371].

Advantages and Disadvantages of Fluidized Beds for Industrial Operations

The fluidized bed has desirable and undesirable characteristics. Table 1 compares its behavior as a chemical reactor with other reactors. Its *advantages* are;

1. The smooth, liquidlike flow of particles allows continuous automatically controlled operations with easy handling.
2. The rapid mixing of solids leads to close to isothermal conditions throughout the reactor; hence the operation can be controlled simply and reliably.

3. The whole vessel of well-mixed solids represents a large thermal flywheel that resists rapid temperature changes, responds slowly to abrupt changes in operating conditions, and gives a large margin of safety in avoiding temperature runaways for highly exothermic reactions.
4. The circulation of solids between two fluidized beds makes it possible to remove (or add) the vast quantities of heat produced (or needed) in large reactors.
5. Heat and mass transfer rates between gas and particles are high when compared with other modes of contacting.
6. The rate of heat transfer between a fluidized bed and an immersed object is high; hence heat exchangers within fluidized beds require relatively small surface areas.

Its disadvantages are;

1. For bubbling beds of fine particles, the difficult-to-describe flow of gas, with its large deviations from plug flow, represents inefficient contacting. This becomes especially serious when high conversion of gaseous reactant or high selectivity of a reaction intermediate is required.
2. The rapid mixing of solids in the bed leads to nonuniform residence times of solids in the reactor. For continuous treatment of solids, this gives a nonuniform product and poorer performance, especially at high conversion levels. For catalytic reactions, the movement of porous catalyst particles, which continually capture and release reactant gas molecules, contributes to the backmixing of gaseous reactant, thereby reducing yield and performance.

The solution of two above disadvantages is using Circulating Fluidized Bed, CFB.

3. Friable solids are pulverized and entrained by the gas and must be replaced.
4. Erosion of pipes and vessels from abrasion by particles can be serious.
5. For noncatalytic operations at high temperature, the agglomeration and sintering of fine particles can require a lowering in temperature of operations, thereby reducing the reaction rate considerably.
6. It is suitable for large-scale operations.

The compelling advantage of overall economy of fluidized contacting has been responsible for its successful use in industrial operations. But such success depends on understanding and overcoming its disadvantages.

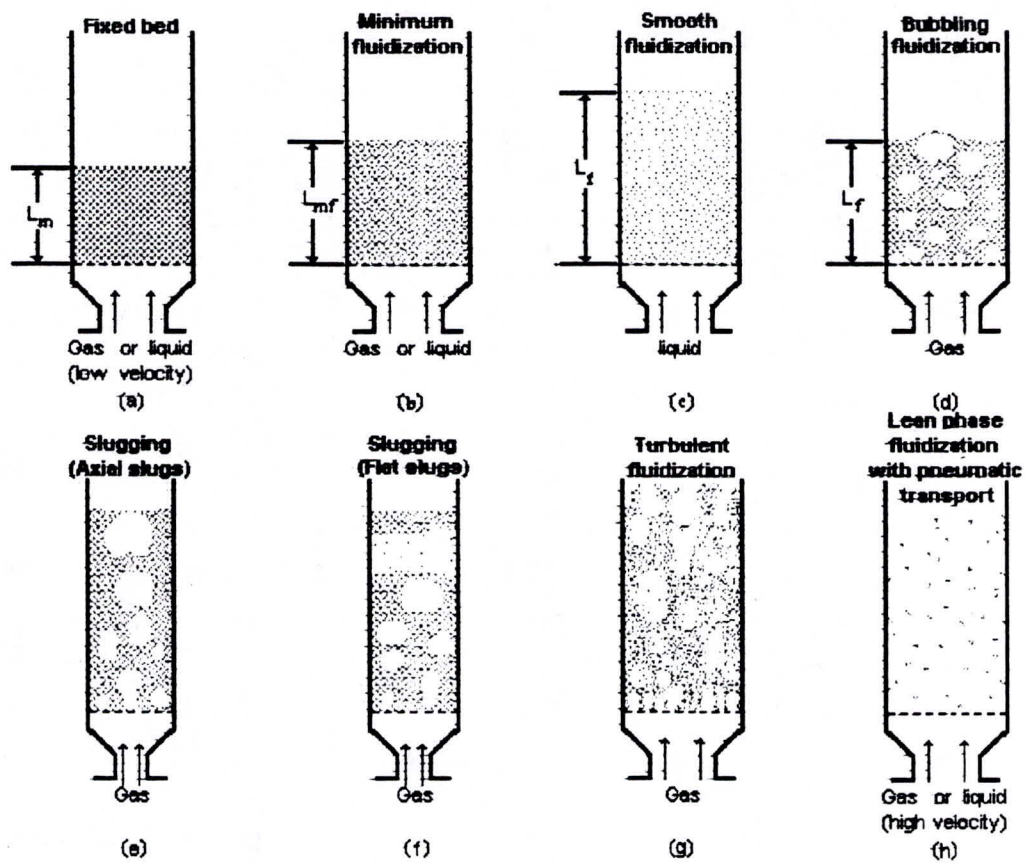


Figure 1 Various kinds of fluidized bed regimes

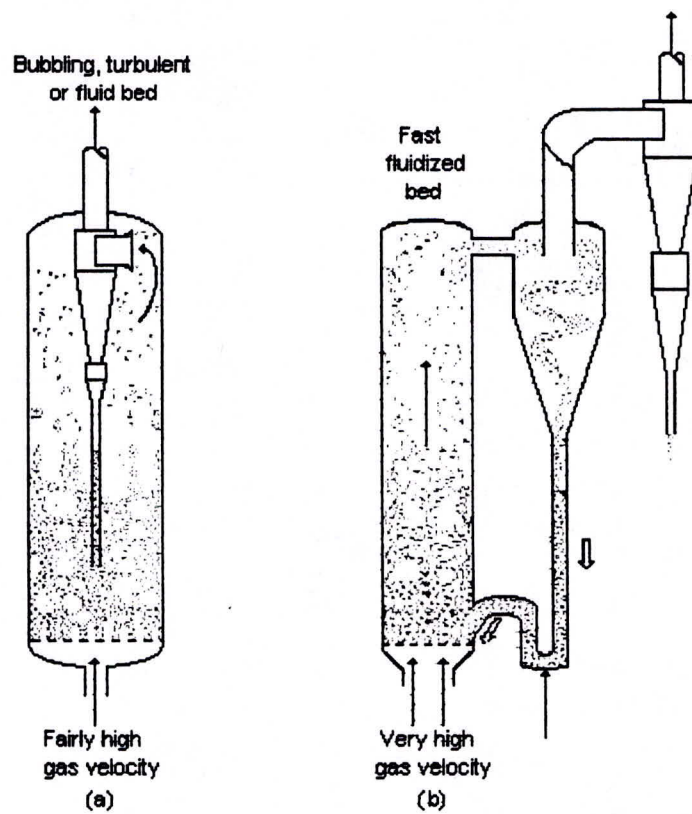


Figure 2 Collecting modes of entrained particles

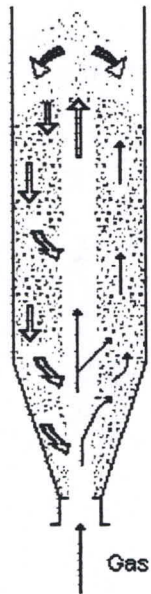


Figure 3 Spouted bed

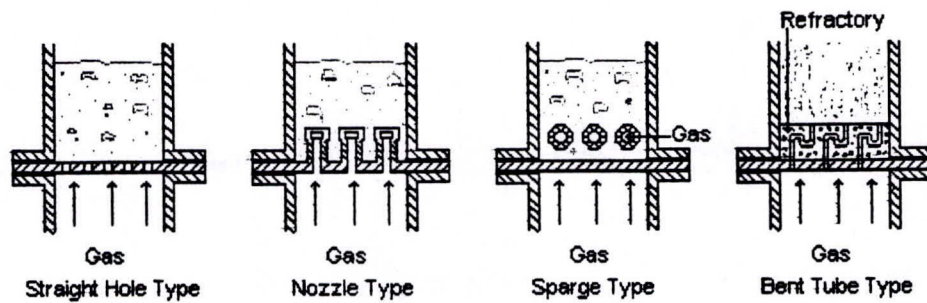


Figure 4 Types of gas distributor plates

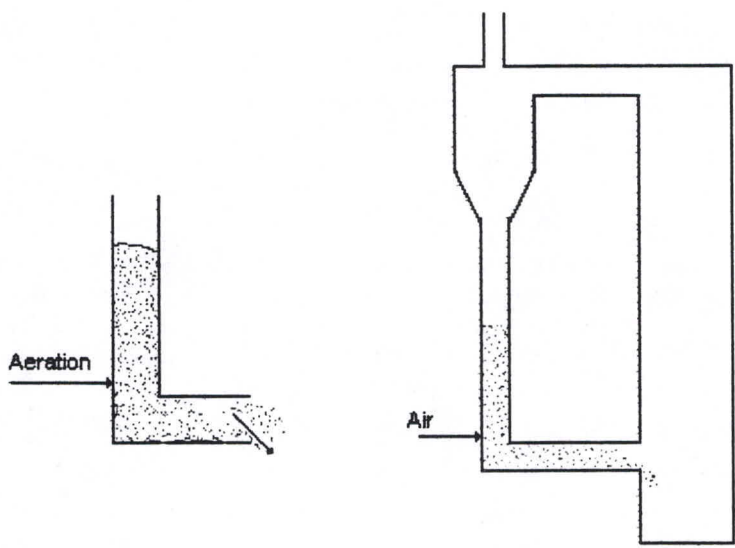


Figure 5 L-valve

	Solid-Catalyzed Gas-Phase Reaction	Gas-Solid Reaction	Temperature Distribution in the Bed	Particles
Fixed Bed	Only for very slow or nondeactivating catalyst. Serious temperature control problems limit the size of units.	Unsuited for continuous operations yield nonuniform product.	Where much heat is involved large temperature gradients occur.	Must be fairly large and uniform. With poor temperature control these may sinter and clog the reactor.
Moving Bed	For large granular rapidly deactivated catalyst. Fairly large-scale operations possible.	For fairly uniform sized feed with little or no fines. Large-scale operations possible.	Temperature gradients can be controlled by proper gas flow or can be minimized with sufficiently large solid circulation.	Fairly large and uniform; top size fixed by the kinetics of the solid recirculation system, bottom size by the fluidizing velocity in reactor.
Bubbling and Turbulent Fluidized Bed	For small granular or powdery nonfriable catalyst. Can handle rapid deactivation of solids. Excellent temperature control allows large-scale operations.	Can use wide range of solids with much fines. Large-scale operations at uniform temperature possible. Excellent for continuous operations, yielding a uniform product.	Temperature is almost constant throughout. This is controlled by heat exchange or by proper continuous feed and removal of solids.	Wide size distribution and much fines possible. Erosion of vessel and pipelines, attrition of particles and their entrainment may be serious.
Fast Fluidized Bed and Cocurrent Pneumatic Transport	Suitable for rapid reactions. Attrition of catalyst is serious.	Suitable for rapid reactions. Recirculation of fines is crucial.	Temperature gradients in direction of solids flow can be minimized by sufficient circulation of solid.	Fine solids, top size governed by minimum transport velocity. Severe equipment erosion and particle attrition.

Table 1 Comparison of Types of Contacting for Reacting Gas-Solids Systems.

RECOMMENDATIONS

During the time I worked at TUNS, there were some problems that I encountered. These problems include:

1. There are a small number of text book in Fluidization; and there is only one copy for each book. So I have many problems for find the information that I looked for.
2. In my opinion, the number of personal computer are not sufficient for the students. Sometimes there is no a personal computer for me to use; because every workstation is possessed.

Two problems that I mentioned are a big problem to solve; because their origin is the money. We must spent a long time for solve these problems. However I really like many systems about the library and the computer room at TUNS.

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Appendix A

The calculation examples about Fluidization

Example 1 Calculation of Minimum Fluidization Velocity

A bed consists of uniform spherical particles of diameter 3 mm and density 4200 kg/m³. What will be the minimum fluidization velocity in a liquid of viscosity 3 mNs/m² and density 1100kg/m³?

Solution

By definition:

$$\begin{aligned}\text{Galileo number } Ga &= \frac{d^3 \rho (\rho_s - \rho) g}{\mu^2} \\ &= \frac{(3 \times 10^{-3})^3 \times 1100 \times (4200 - 1100) \times 9.81}{(3 \times 10^{-3})^2} \\ &= 1.003 \times 10^5\end{aligned}$$

Assuming a value of 0.4 for e_{mf} ,

$$\text{from } (Re'_{mf})_{e_{mf}=0.4} = 25.7 \times \left\{ \sqrt{1 + 5.53 \times 10^{-5} Ga} - 1 \right\}$$

$$\begin{aligned}(Re'_{mf})_{e_{mf}=0.4} &= 25.7 \times \left\{ \sqrt{1 + (5.53 \times 10^{-5})(1.003 \times 10^5)} - 1 \right\} \\ &= 40\end{aligned}$$

$$\begin{aligned}u_{mf} &= 40 \times \frac{3 \times 10^{-3}}{3 \times 10^{-3} \times 1100} \\ &= 0.0364 \text{ m / s} \\ &= 36.4 \text{ mm / s}\end{aligned}$$

Ans.

It is specified that the volume flow rate of air is $0.2 \text{ m}^3 / \text{sec}$. Assume the operating conditions is atmospheric pressure and the temperature is 800°C , so the air can be treated as an ideal gas since it is at a high temperature and low pressure relative to its critical values ($T_{cr} = -147^\circ\text{C}$ and $P_{cr} = 2290 \text{ kPa}$ for nitrogen, the main constituent of air).

To determine the mass flow rate of air, we need to find density of the air first. This is determined from the ideal-gas relation at the inlet conditions:

$$\begin{aligned}\rho &= \frac{P}{RT} = \frac{101.325}{0.287 \times 1073} \\ &= 0.329 \text{ kg} / \text{m}^3\end{aligned}$$

Then

$$\begin{aligned}\dot{m}_{air} &= \rho \times \dot{V} \\ &= 0.329 \times 0.2 \\ &= 0.0658 \text{ kg} / \text{sec}\end{aligned}$$

Also, the fuel flow rate is determined by

$$\begin{aligned}AF &= \frac{\dot{m}_{air}}{\dot{m}_{fuel}} = 8.469 \\ \dot{m}_{fuel} &= \frac{\dot{m}_{air}}{8.469} \\ &= 7.769 \times 10^{-3} \text{ kg} / \text{sec}\end{aligned}$$

That is, $7.77 \times 10^{-3} \text{ kg}$ of lignite is supplied every second during this combustion process.

b) The theoretical sorbent requirements are 3.15 kg of limestone per kg sulfur in the fuel [Feridun Hamdullahpur & V. Ismet Ugursal].

Recall that

$$\dot{m}_{fuel} = 7.769 \times 10^{-3} \text{ kg} / \text{sec}$$

The percentages of sulfur in lignite is 1%, so the mass flow rate of sulfur is determined by

$$\begin{aligned}\dot{m}_{sulfur} &= 7.769 \times 10^{-3} \times 0.01 \\ &= 7.769 \times 10^{-5} \text{ kg} / \text{sec}\end{aligned}$$

And the mass flow rate of limestone is determined by

$$\begin{aligned}\dot{m}_{\text{limestone}} &= 7.769 \times 10^{-5} \times 3.15 \\ &= 2.447 \times 10^{-4} \text{ kg / sec}\end{aligned}$$

That is, 2.45×10^{-4} kg of limestone is supplied every second during this combustion process.

- c) The size of fuel particles do not necessarily have a major bearing on that of bed materials, because they constitute only a minor fraction (1-3%) of the total bed materials in the CFB furnace. (from Prabir Basu, Scott A. Fraser, *Circulating Fluidized Bed Boilers: Design and Operations*, Page 6, Butterworth-Heinemann, Boston, 1991.)

Assume there are 2% of lignite in bed materials, so the mass flow rate of bed materials is determined by

$$\dot{m}_{\text{fuel}} = 7.769 \times 10^{-3} \text{ kg / sec}$$

So

$$\begin{aligned}\dot{m}_{\text{bed materials}} &= \frac{98}{2} \times 7.769 \times 10^{-3} \\ &= 3.807 \times 10^{-1} \text{ kg / sec}\end{aligned}$$

That is, 3.81×10^{-1} kg of bed materials is supplied every second during this combustion process.

Ans.

Appendix B

The example of figures of experiment in Fluidization

Name: Levent Ersoy

Name of Thesis: The effect of Secondary air injection on the Hydrodynamics of Circulating Fluidized Beds

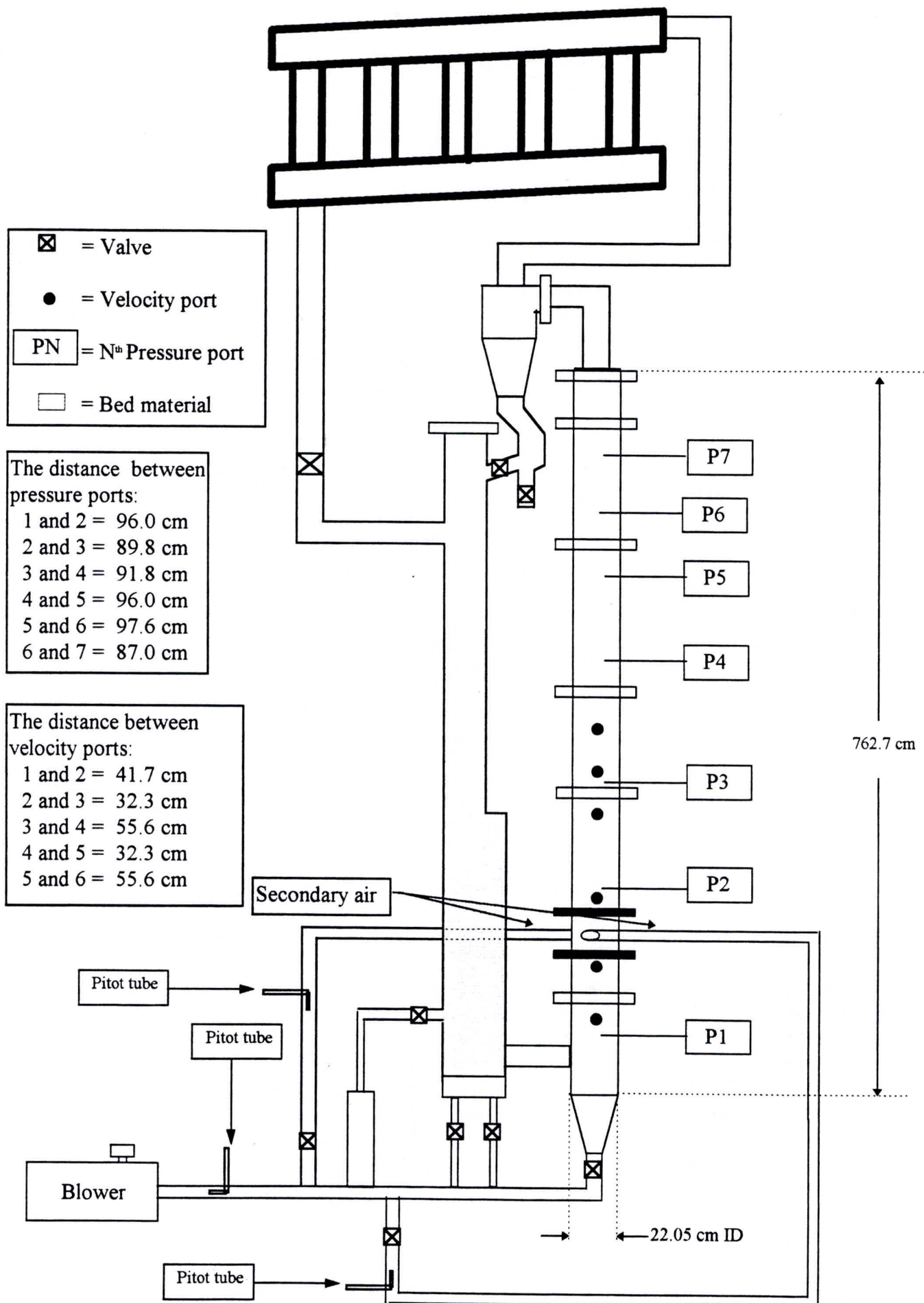
Descriptions:

Differential pressures are measured by pressure transducers.

Particle velocity is measured by fiber optical velocity probe (Vector).

Fluid flow rates are measured by using pitot tubes.

Figure 1: The whole experiment set-up



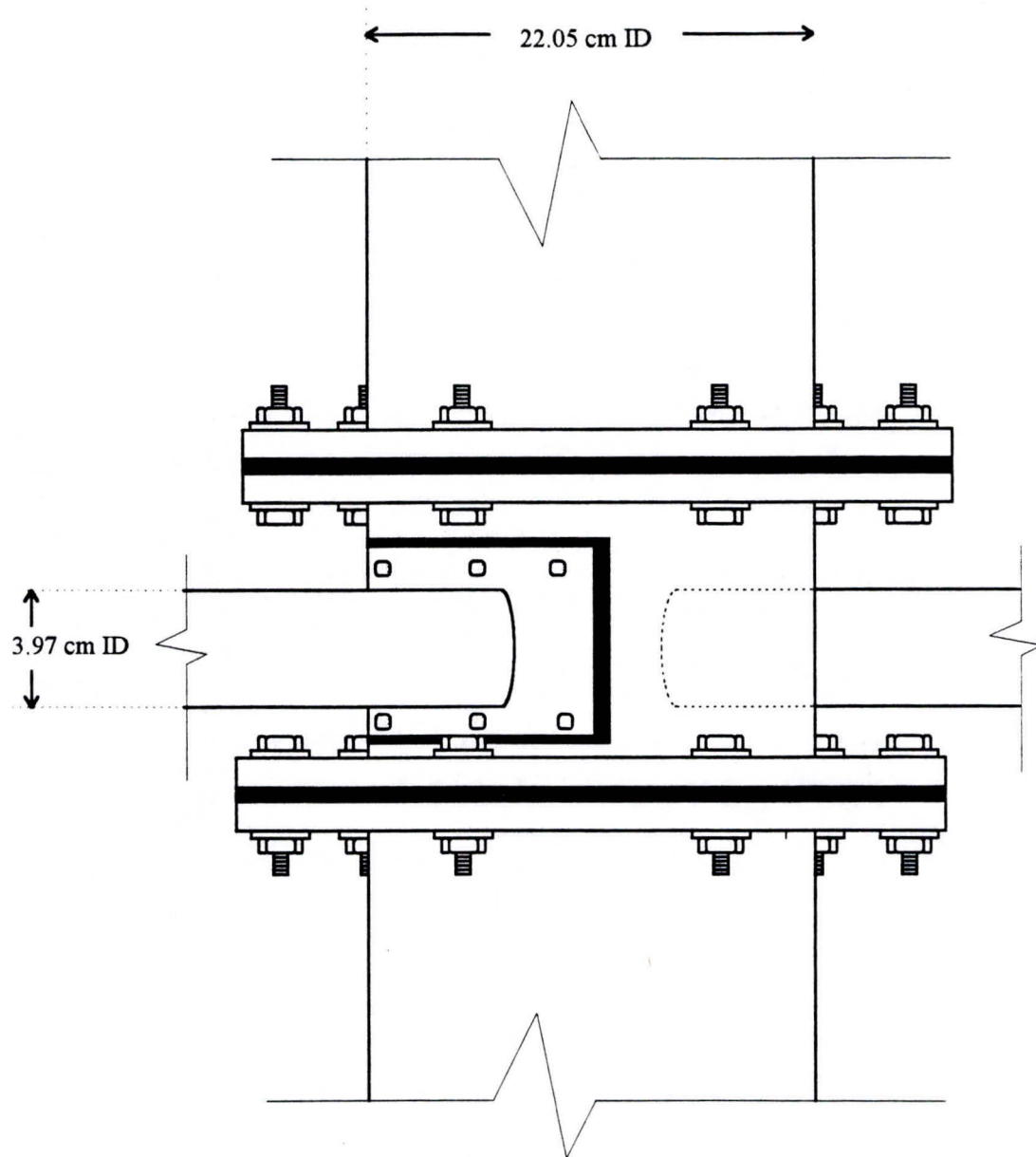


Figure 2 Injector column with secondary air port

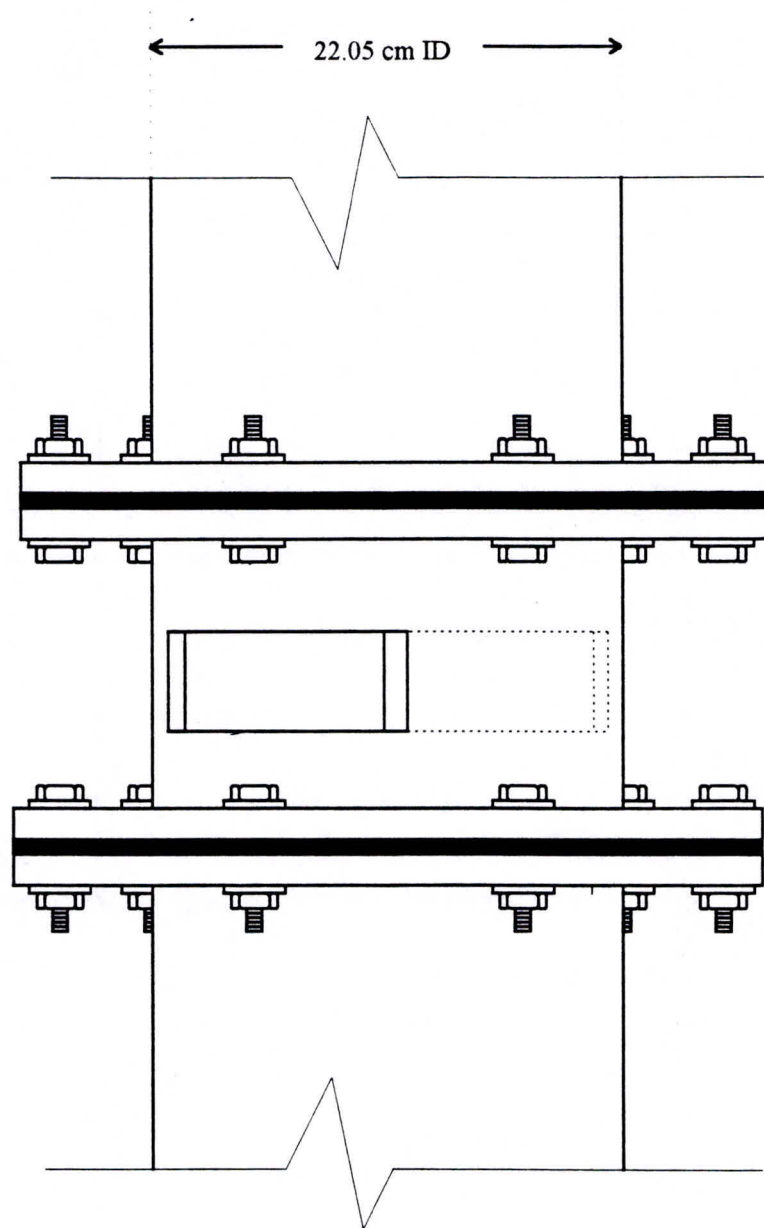


Figure 3 Injector column without secondary air port

Jan.21, 1997

TO WHOM IT MAY CONCERN

Ms. H. Miliwat worked as a research assistant in my lab for a period of 4 months ending in Dec. 28, 1996. During this period she undertook several tasks related to literature search, data analysis and presentation, as well as an experimental investigation of the dynamic surface tension of industrial streams.

I found her to be an exceptionally intelligent and hard working person. Through her perseverance, she was able to solve some very difficult problems encountered during her experimental work. She is also a very pleasant person to work with and her communication skills are good in spite of the short exposure to an English language environment. I strongly recommend her for an industrial career or as a graduate student.



A.M. Al Taweel
Professor