

## ANAEROBIC DIGESTION TREATMENT OF CHEESE WHEY FOR PRODUCTION OF METHANE IN A TWO STAGE UPFLOW PACKED BED REACTOR.

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### ABSTRACT:

Anaerobic Digestion has become a competent technology for biological conversion of organic content of Dairy wastewater. The dairy industry generates whey which is the most important wastewater produced, with an extremely high organic load(50-70 g/lit COD). Cheese whey as a by-product of the dairy industry consists of carbohydrates mainly lactose, lactic acid, proteins and fat, etc. In anaerobic Digestion process whey is initially hydrolyzed and converted to organic acids by acidogenic bacteria then the degradation is followed by the methanogenic bacteria. The anaerobic process has a number of advantages; one of them is the production of methane as an energy source which consists of 50 to 70 % methane. In this Study the novel systems of two stage upflow anaerobic packed bed reactor was used to reduce HRT for better conversion & avoid wash out of cell. Two operating schemes for HRT were maintained and methane gas, volatile fatty acids, COD removal efficiency were calculated.

**Keyword:** Cheese Whey, Anaerobic process, Acidogenic & Methanogenic bacteria, UAPB Reactor, COD (Chemical Oxygen Demand), HRT (Hydraulic Retention Time), volatile fatty acids (VFA).

### [I] INTRODUCTION

Dairy wastes are plentiful in dairy industries. The wastes contain high organic matters and the disposal of the effluents may cause severe environmental pollution. The dairy industry generates residues from which whey is the most important wastewater produced, with an extremely high organic load. Whey is a byproduct of cheese and casein manufactures; mainly consists of carbohydrates lactose, salts, lactic acid, proteins and fat, respectively [9, 15, 22] It has a high biological oxygen demand which

is caused by its protein and carbohydrate content and it creates a problem when disposed as wastewater. The worldwide production of fluid whey by the cheese and casein industries runs into millions of tons, and yet effective utilization of this material is not well developed. World annual production of whey is estimated to be 115 million tons; approximately 47 % of the produced whey is disposed into the environment [2]

Anaerobic operation has recently been accepted as an effective mean of treating wastewaters. For wastewater with a total BOD in excess of 4000 mg·l<sup>-1</sup> an anaerobic contact process was cheaper than an aerobic process.<sup>[6,19]</sup> The some benefits of anaerobic processes in treating organic wastewater are given as: This process requires only relatively few inorganic nutrients for treating wastewater and the major gas product is methane, a valuable and sustainable energy resource. No requirement of oxygen in the process, in contrary strictly anaerobic conditions and the absence of nitrate are required. Produce a residual sludge which is inoffensive and useful as a soil conditioner and low grade fertilizer.<sup>[3]</sup>

Beside the benefits, there are also some disadvantages of anaerobic processes are reported such as, The major microbial population is inherently unstable, thereby requiring that the system must be kept under close supervision and control and the maximum specific growth rates of the organisms are relatively low.

Anaerobic degradation process can be separated into two phases. The 'acid fermentation' phase, leads to the production of intermediate products predominated by the volatile organic acids; and the 'methane fermentation' phase, resulting in the conversion of these intermediates to stable end products, principally methane and carbon dioxide.<sup>[6, 17]</sup> For the two phase reactor system it is necessary that first reactor contain maximum number of hydrolytic, acidogenic & acetogenic bacteria and second reactor contain maximum number of methanogenic bacteria.<sup>[21,26]</sup>

The first phase is different from the second one in bacterial varieties, digestion rate, environmental demands, degradation process and products. The introduction of an acidogenic phase should enable optimization of the conditions required for many of complex organic chemicals present in a wastewater to be converted to short-chain volatile fatty acids (VFA) and other simple compounds. This, in turn, buffers the slow-growing methanogens,

predominantly present in second phase reactor, from possible toxins or inhibitors and ensures a uniform feed stock for the methanogens.<sup>[1,13]</sup> Pohland et al have investigated the merits of two-phase anaerobic degradation (TPAD) for acidogenesis of sewage sludge in order to achieve a significant improvement in sludge treatment efficiencies.<sup>[23]</sup> Since this initial investigation into the TPAD, considerable research has been carried out with many high-strength industrial wastewaters to assess the advantages of this treatment over conventional single-phase systems.<sup>[14]</sup>

Packed bed used in the reactor give surface area for attachment to growth of bacteria. This increases cell concentration in reactor & prevent wash out of cell from the reactor. Thus two phase upflow packed bed reactor can be used for treatment of whey.<sup>[12, 20]</sup>

## [II] MATERIALS AND METHODS

### 2.1) Cheese Whey:

Cheese whey is the waste (by product) produced in Cheese making. It contains high organic material and it contributions 95% COD for total whey produced in dairy industry.<sup>[12]</sup> In this research work whey was collected from "SHRI WARANA SAHAKARI DUDH UTPADAK PRAKRIYA SANGH LIMITED, KORENAGAR" Tal. Panhala, Dist. Kolhapur, as per requirement & stored at cool temperature to avoid unwanted reactions. Analysis of whey collected has done from Nikhil Laboratory, Sangli. The analysis report of composition of whey is as per the table 1.

[Table 1]:

Sr No.	Content	Concentration
1	pH	6-6.5
2	Total Solids	4.6 %
3	Lactose Concentration	2.1 %
4	Fat	1.22 %
5	COD	10000 mg/ml
6	Standard plate count	200× 10 <sup>4</sup>

**Table: 1** Composition of cheese whey from Warana dairy

**2.2) Inoculums:**

Inoculum is the bioculture used for start up of reactor. For anaerobic reactor inoculums volume is around 10-20% of the total volume of reactor. [26] 10 liters of bioculture as inoculum were collected from the outlet of biogas plant of Warana sugar industry.

**2.3) Laboratory scale UAPB Reactor Design:**

After detail literatures study of anaerobic digestion and analyzing cheese whey properties, the two stage anaerobic digester were proposed. [13, 17, 1] i.e. Two Stage Upflow Packed bed Reactor for treatment of whey water. At the start of work the proposed design had drawn as shown in figure 1. It consist of feed tank, two small digester (Reactor 1 & Reactor 2) with packing material, effluent collecting tank, gas collector etc. The flow rate of material inside the reactor was maintained by using height difference between reactors and Tank.

**a) Feed tank:**

Feed tank used as reservoir of feed. It is plastic container of 10 liter volume, which was maintained at height 8 feet from the ground. The ball valve used to control flow rate.

**b) First Reactor:**

It is a first anaerobic packed bed reactor contains small packing material. Reactor contained 10 liter volume of liquid in it. It is maintained at height 5 feet from ground. We made reactor by using PVC pipe of diameter 160 mm & 700 mm in height. The both end of PVC pipe closed by end caps of suitable diameter.

Hydrolysis, Acidogenesis & Acetogenesis reactions were carried in first reactor. It have three connections one at the bottom of reactor to connect it to feed tank. Second one at the middle of reactor as sample port to check pH & temperature of reacting material.

**c) Second Reactor:**

It also made up of PVC pipe of diameter 200 mm & 900 mm in height. It was second anaerobic packed bed reactor also contain packing material. This reactor was used for growth of methanogenic bacteria. It has larger volume than first reactor so get large population methanogenic bacteria. It has 20 liter liquid volume plus 2 liter head space for collection of gas.

**d) Sample port:**

Sample port connection taken at middle of the each reactor. It was used to collect the sample for checking of Different parameter which affect on anaerobic digestion.

**e) Effluent tank:**

It is plastic container of 10 liter volume placed at the ground level. It was connected at outlet second reactor for the collection of effluent from reactor.

**f) Packing material:**

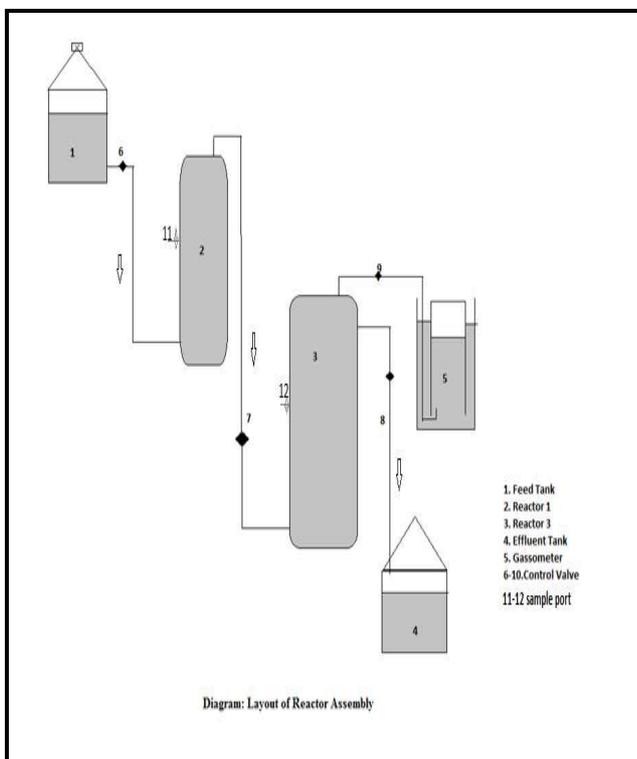
We used small pieces of PVC pipe of 16mm diameter & length of 40-50 mm as packing material. We made scratches on the outer side of packing material for the maximum biofilm formation on it.

**g) Valves:**

Plastic ball valves were used for control flow between feed tank & reactors. Metal ball valve used for control gas flow. Small size of plastic valves used at sample port.

**h) Gas collector:**

It is plastic bottle of 500 ml volume & glass



**Fig. 1.** Design of Two Stage Upflow Anaerobic Packed Bed Reactor

container of 2 liter. It was used to measure gas volume by water displacement principle. The Laboratory scale UAPB Reactor after fabrication was effectively used to carry out this work. The working model UAPB Reactor is as shown in figure 2.



**Fig: 2.** Working Model of Two Stage UAPB Reactor

**2.4) Start-up of Reactor:**

Start-up is the process to increase biomass growth in the reactor. For the start-up of reactor it was inoculated with bioculture and substrate by providing optimum condition for rapid growth of microorganisms. The source and size of seed sludge during the initial mode of operation are the most critical factors for start-up of anaerobic digesters. For an effective start-up, seed sludge was selected with high levels of methanogens present in it.

For start-up we had inoculated first Reactor with two liter inoculums & 1 liter of whey. From second day 1 liter whey added every day. When first reactor completely filled second reactor inoculated 5 liter of inoculums & each day 1 liter of outlet from first reactor till second reactor completely filled. For next 3 days no any further addition was made in reactor.

During start up of reactor optimum pH & temperature maintained for rapid growth of bacteria.

**2.5) Reactor Operation:**

After completion of start-up period of both reactors, the objective was to maintain two different HRT for reactors. First HRT was of 4 day for first reactor & 8 day for second reactor. Second HRT was of 3 day for first reactor & 6 day for second reactor. The five reading were noted for first HRT and second HRT.

**2.6) Analytic Methods:**

The analytic values were calculated for respective sample by using following methods: Chemical Oxygen Demand (COD) was determined by Potassium dichromate reflux method and Volatile Fatty Acids (VFA) concentration was determined by Distillation method. The composition of biogas was calculated by CO<sub>2</sub> scrubbing method and volume of Biogas was calculated by water displacement method. [24, 25]

**[III]RESULT AND DISCUSSION**

**3.1 HRT: 12 (4+ 8) Days**

**A) COD Removed and Methane Produced**

After completion of desired HRT (12 Days) two times biogas analyzed for methane percentage. Its percentage was 50% & 52 % respectively. So 52 % considered for calculation methane content. The details of COD removal and production of methane are reported in Table 2.

**[Table: 2]**

Day	Effluent COD mg/lit.	Efficiency of COD Removal (%)	Total biogas Produced (ml)	Methane produced (ml)
1	310	96.90	280	145.6
2	336	96.64	320	166.4
3	340	96.60	340	176.8
4	360	96.40	370	192.4
5	350	96.50	420	218.4

**Table 2:** (4+8 Days): COD Removal & Methane Produced at HRT

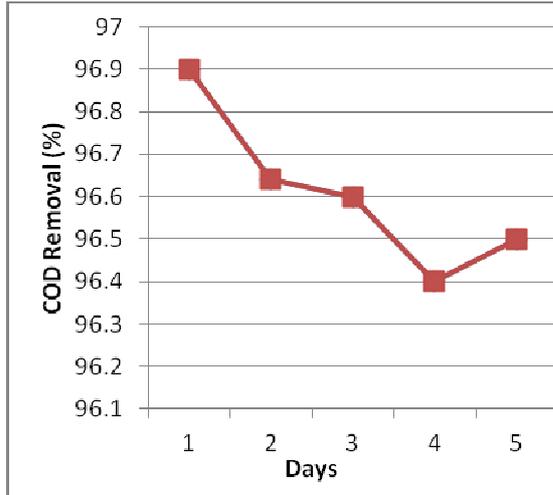
The maximum methane production obtained was 218.4 ml on 5<sup>th</sup> day.

**B): Volatile Fatty Acids:**

[Table 3]

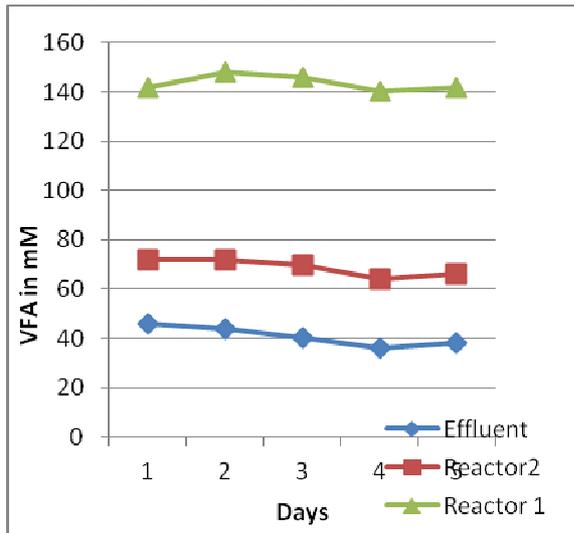
Day	Reactor 1 (mM)	Reactor 2 (mM)	Effluent (mM)
1	142	72	46
2	148	74	44
3	146	70	40
4	140	64	36
5	142	66	38

**Table: 3** Volatile Fatty Acid in (mM) concentration at HRT (4+8 Days)



**Figure: 3.** Graph of COD Removal (%) Vs Time (Days)

The graph (Fig: 3.) shows the efficiency of reactor for 5 days. It ranges from 96.5 % to 96.9 %. So at this HRT process have capacity to remove about 96.5 % of COD from the reactor.



**Fig: 4.** Graph Volatile Fatty Acids Vs Days

As shown in graph volatile fatty acid produced in first reactor with concentration of about 145 mM. This VFA had been consumed in the second reactor. The final concentration VFA in Effluent was about 40 mM.

**3.2 HRT: 9 (3+6) Days:**

[Table: 4]

Days	Effluent COD (mg/liter)	Efficiency (%)	Total biogas produced (ml)	Methane produced
1	600	94	550	275
2	550	94.5	580	290
3	450	95.5	620	310
4	600	94	610	305
5	550	94.5	630	315

**Table: 4** COD Removal and Methane Produced at HRT 9 (3+6) days

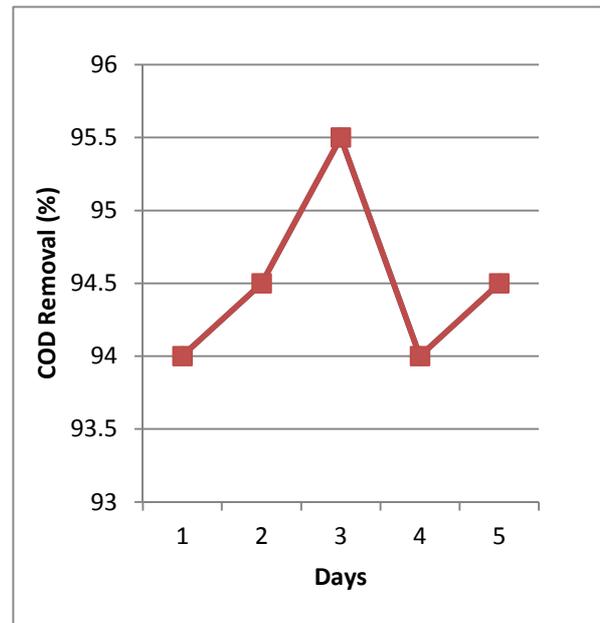
After completion of desired HRT (9 days) biogas sample analyzed two times to determine methane percentage. It was 50 % for both time, thus 50% considered for calculation methane content. The details of COD removal and production of methane are reported in Table 4.

The maximum methane production obtained was 315 ml on the 5<sup>th</sup> Day.

[Table: 5]

Days	Reactor 1 (mM)	Reactor 2 (mM)	Effluent (mM)
1	186	92	56
2	178	96	52
3	180	86	60
4	182	88	48
5	180	90	50

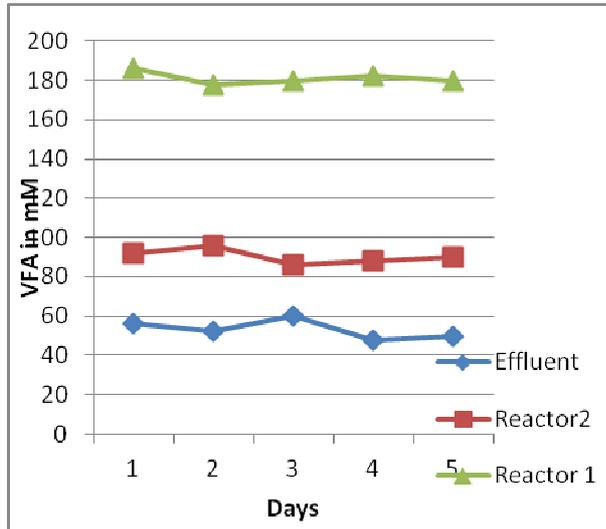
**Table: 5.** Volatile Fatty Acid in (mM) concentration at HRT (3+6 Days)



**Fig: 5.** Graph of COD Removal (%) Vs Time (Days)

The graph shows the efficiency of reactor for 5 days. It ranges from 94 to 95.5 %. So at this HRT process have capacity to remove about 95 % COD from the whey.

**B) Volatile Fatty Acids:**



**Fig: 6.** Graph of Volatile Fatty Acids Vs Days

As shown in graph (Fig:6) volatile fatty acid produced in first reactor with concentration of about 180 mM .This VFA had been consumed in the second reactor. The final concentration VFA in Effluent was about 50 mM.

**CONCLUSION**

The present study shows that two stage upflow anaerobic packed bed reactor can be used for treatment of cheese whey. In this system it is possible to maintain optimum growth of hydrolytic & methanogenic bacteria separately. The results of this work have shown that the use of Upflow reactor to achieve high COD removal. Depending on HRT in operation of the reactor, it can be used to obtain 94% to 96 % COD removal efficiency. Two phase digestion with pH and temperature control results in a higher biogas production rate with cheese whey wastewater digestion (K.V. Rajeshwari et al.) So by optimizing the process the application of this system can be used for dairy waste treatment of cheese whey.

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**REFERENCES**

- 1) Tawfika, M. Sobheyb, M. Badawya (2008) Treatment of a combined dairy and domestic wastewater in an up-flow anaerobic sludge blanket (UASB) reactor followed by activated sludge (AS system). *Desalination* 227 167–177
- 2) Andrés Illanes (2011) Whey upgrading by enzyme biocatalysis. *Electronic Journal of Biotechnology*. vol14-issue6-fulltext-11
- 3) Aydin, A. F.; Ersahin, M. E.; Dereli, R. K.; Sarikaya, H. Z. & Ozturk, I. (2010). “Long term Anaerobic treatability studies on opium alkaloids industry effluents”. *Journal of Environmental Science & Health, Part A: Toxic Substances & Environmental Engg.* 192- 200.
- 4) Azbar, N.; Bayram, A.; Filibeli, A.; Muezzinoglu, A.; Sengul, F. & Ozer, A. (2010). “A review of waste management options in olive oil production”.*Critical Reviews in Environmental Science & Technology Engg.* 209-247.
- 5) Baspinar A.B. (2008). “Hydrogen Sulphide Removal from Biogas With Nitrate Coming from An Industrial Wastewater Treatment Plant”.
- 6) Batstone, D. J.; Keller, J.; Angelidaki, I.; Kalyuzhnyi, S. V.; Pavlostathis, S. G.; Rozzi, A.; Sanders, W. T. M.; Siegrist, H. & Vavilin, V. A. (2002). “Anaerobic Digestion Model No.1”, *Water Science and Technology* Vol 45 No 10 pp 65–73
- 7) Beal, L. J. & Raman, D. R. (2000). “Sequential Two-Stage Anaerobic Treatment of Confectionery Wastewater”. *Journal of Agricultural Engineering Research* 76, 211-217
- 8) Benedict, S. R. (1 December 1908).” A Reagent For the Detection of Reducing Sugars”.*J. Biol. Chem.* 485-487.
- 9) Boening, P.H., Larsen, V.F., 1982. Anaerobic fluidized bed whey
- 10) Cansu Filik İscen, Semra İlhan, M. Ercengiz Yildirim (2004) - “Anaerobic reatability and

- methane production potential of industrial waste water”. Eskisehir. Eng & Arch. Eskisehir Osmangazi University vol No. 2
- 11) Eriks Skripsts, Vilis Dubrovskis, Janis Jasko, Eduards Zabarovskis, Vladimirs Kotelenecs(2011)- “Investigation of biogas production of cheese whey in processing with ozone before anaerobic digestion”. Engineering for Rural development jelgava 26.
  - 12) G. D. Najafpour, M. Komeili, M. Tajallipour, and M. Asadib( 2009)- “Bioconversion of Cheese Whey to Methane in an Upflow Anaerobic Packed Bed Bioreactor”. Cem.Biochem.Q-24. 111-117.
  - 13) G.D. Najafpour, B.A. Hashemiyeh, M. Asadi and M.B. Ghasemi(2008)- “Biological Treatment of Dairy Wastewater in an Upflow Anaerobic Sludge-Fixed Film Bioreactor”. American –European J. Agri c. & Environ.sci.251-257.
  - 14) Herbert H.P. Fang (2005) – “Applications of two-phase anaerobic degradation in industrial wastewater treatment”. Int. J. Environmental & pollution vol 23.
  - 15) Jeffrey E. Fehrs, P.E Williston, Vermont. (2000) “Report On-Vermont methane pilot project resource assessment”.
  - 16) Joe Kramer, Senior Project Manager, Energy Center of Wisconsin (2011) – “Great Lakes Region Food Industry Biogas Casebook”. Energy Center Of Wisconsin Report 261-1.
  - 17) K.V. Rajeshwari, M. Balakrishnan, A. Kansal, Kusum Lata, V.V.N. Kishore. (1999) – “State-of-the-art of anaerobic digestion technology for industrial wastewater treatment”. Renewable & sustainable Energy Review 4,135-156.
  - 18) Katerina Stamatelatos, Georgia Antonopoulou, Asimina Tremouli, and Gerasimos Lyberatos(2011)- “Production of Gaseous Biofuels and Electricity from Cheese Whey”. Ind. Eng. Chem. Res. 50, 639-644.
  - 19) Marisa Hendajani, MT. Prof Dr.rer.nat.J.Winter, Prof Dr. – Ing. E.h.H.H.Hahn, PHD, Karlsruhe University (2004) –“Degradation of Whey in an Anaerobic Fixed Bed (AnFB) Reactor”.
  - 20) Mi JungPark, JiHyeJo, DongheePark, DaeSungLee, JongMoonPark(2010) – “Comprehensive study on a two-stage anaerobic digestion process for the sequential production of hydrogen and methane from cost-effective molasses”. International Journal of Hydrogen Energy vol. 35, no. 12, 6194-6202.
  - 21) Mustafa Evren Ersahin, Hale Ozgun,Recep Kaan Dereli and Izzet Ozturk Istanbul Technical University, Turkey- “Anaerobic Treatment of Industrial Effluents: An Overview of Applications”.
  - 22) Nupur Goyal and D.N. Gandhi (2009) “Comparative Analysis of Indian Paneer and Cheese Whey for Electrolyte Whey Drink”. World Journal of Dairy & Food science.70-72.
  - 23) Pohland, F.G., Ghosh, S. (1971) Developments in anaerobic stagilization of organic wastes—the two-phase concept. Env. Letters, 1, 4, 255-266.
  - 24) Richard L. Zollars(2010) –“Chemical Process Principles and Calculations”.
  - 25) Robert D. Simoni, Robert L. Hill, and Martha Vaughan (2002). “Benedict's Solution, a Reagent for Measuring Reducing Sugars”. J. Biol.Chem.10-11.
  - 26) Stina Asplund (2005) – “The Biogas Production Plant at Umeå Dairy Evaluation of Design and Start-up treatment”. Biotechnol. Bioeng. 14, 2539–2556.