Appendix 1 – Reagents, Equipment and Suppliers

Supplier	Chemical
Merck (South Africa)	Ethanol (Absolute), K_2HPO_4, $MgCl_2 \cdot 6H_2O$, $FeSO_4 \cdot 7H_2O$, $CoCl_2 \cdot H_2O$, H_2SO_4, NaCl, Yeast Extract, Peptone, Lactose, Glucose, Agar, MacConkey Agar with Crystal Violet, Phenol red
BDH (Merck, South Africa)	$MnSO_4 \cdot 4H_2O$, $CuSO_4 \cdot 5H_2O$, KCl, Ferric citrate, Sodium Thiosulphate
Associated Chemical Enterprises	Hydrochloric Acid (32%), Granular Activated Carbon
Protea Industrial Chemicals (South Africa)	NH_4HCO_3, $NaHCO_3$, NaOH flakes,
Oxoid	Eosin Methylene Blue Agar, Potato Dextrose Agar, Lab Lemco' powder
Fluka	Resorcinol
Makro (South Africa)	Sucrose

Supplier	Equipment
Measurement and Control Solutions	*DataTaker* DataLogger
Perspex World	Feed Bins, Reservoir
Mazey's Plastics	Perspex for reactor construction
Leeways Garden Centre	Plumbing components
A-Z Technical Services	Electrode housing vessel
Wirsam Scientific	Autoclude Peristaltic Pumps
Swiss Lab	Barometer,
AP Electronics	Wiring, Resistors, Control System Housing
Aquapump	Boyser AMP-16 Peristaltic Pump
Laboratory Automation and Control	Grant Digital 60 Waterbath
Evna Industrial Products	Silicon Grease

Appendix 2 – Media and General Reagents

Unless otherwise specified, all media was made up to 1L with distilled H_2O and autoclaved at 121^oC for 15 minutes.

KLIGLER IRON AGAR

Formula	g/L
Lab Lemco' powder	3.0
Yeast extract	3.0
Peptone	20.0
NaCl	5.0
Lactose	10.0
Glucose	1.0
Ferric citrate	0.3
Sodium thiosulphate	0.3
Phenol red	0.05
Agar	12.0
pH 7.4 ± 0.2	

MODIFIED MACCONKEY BROTH

Formula	g/L
Peptone	20.0
Lactose	10.0
Bile salts	5.0
NaCl	5.0
Neutral red	0.075
Ferric Citrate	1.5
Sodium Thiosulphate	5.0
pH 7.4 ± 0.2	

EOSIN METHYLENE BLUE AGAR (MODIFIED) LEVINE

Formula	g/L
Peptone	10.0
Lactose	10.0
K_2HPO_4	2.0
Eosin Y	0.4
Methylene blue	0.065
Agar	15.0
pH	6.8 ± 0.2

INDOLE MEDIA

Formula	g/L
Tryptone	10.0
NaCl	5.0
Glucose	1.0
Agar	1.25

ENDO FORMULATION

NH_4HCO_3 and $NaHCO_3$ were both filter sterilised due to their low thermal decomposition temperatures. Sucrose and salts were autoclaved together at 121^{o}C for 15 minutes.

Chemical Component	(g/L)
Sucrose	17.80
NH_4HCO_3	5.240
$NaHCO_3$	6.720
K_2HPO_4	0.125
$MgCl_2 \cdot 6H_2O$	0.100
$MnSO_4 \cdot 4H_2O$	0.015
$FeSO_4 \cdot 7H_2O$	0.025
$CuSO_4 \cdot 5H_2O$	0.005
$CoCl_2 \cdot H_2O$	1.24×10^{-4}

MODIFIED ENDO FORMULATION – REACTOR MEDIUM

The components for the reactor were not sterilized due to the volume of chemicals used in the concentrated feed. The final volume of the concentrated feed was 5x with a volume of 100L.

Chemical Component	(g/L)	Concentrated Feed (g/100L)
Sucrose	17.65	8820
NH_4HCO_3	3.490	1732
K_2HPO_4	0.699	3360
$NaHCO_3$	6.720	349.3
$MgCl_2 \cdot 6H_2O$	0.100	50
$MnSO_4 \cdot 4H_2O$	0.015	7.5
$FeSO_4 \cdot 7H_2O$	0.025	12.5
$CuSO_4 \cdot 5H_2O$	0.005	2.5
$CoCl_2 \cdot H_2O$	1.24×10^{-4}	0.062

NUTRIENT BROTH

Component	g/L
Meat Extract	1.0
Yeast Extract	2.0
Peptone	5.0
Sodium Chloride	8.0

Appendix 3

Design of Fluidised Bed Reactor

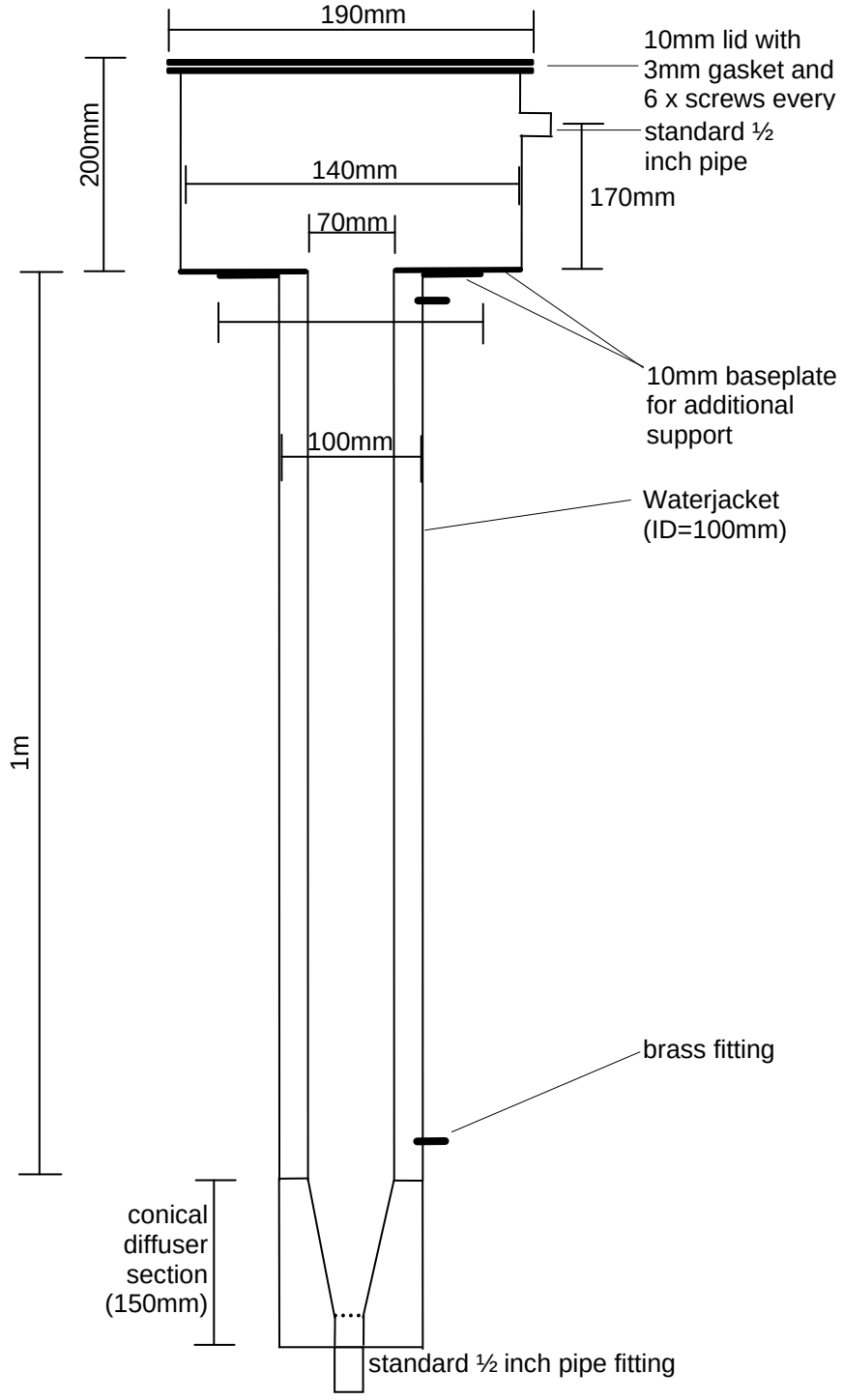

Figure c1: Technical schematic of entire fluidised bed reactor. Reactor was constructed from 5mm perspex unless otherwise stated.

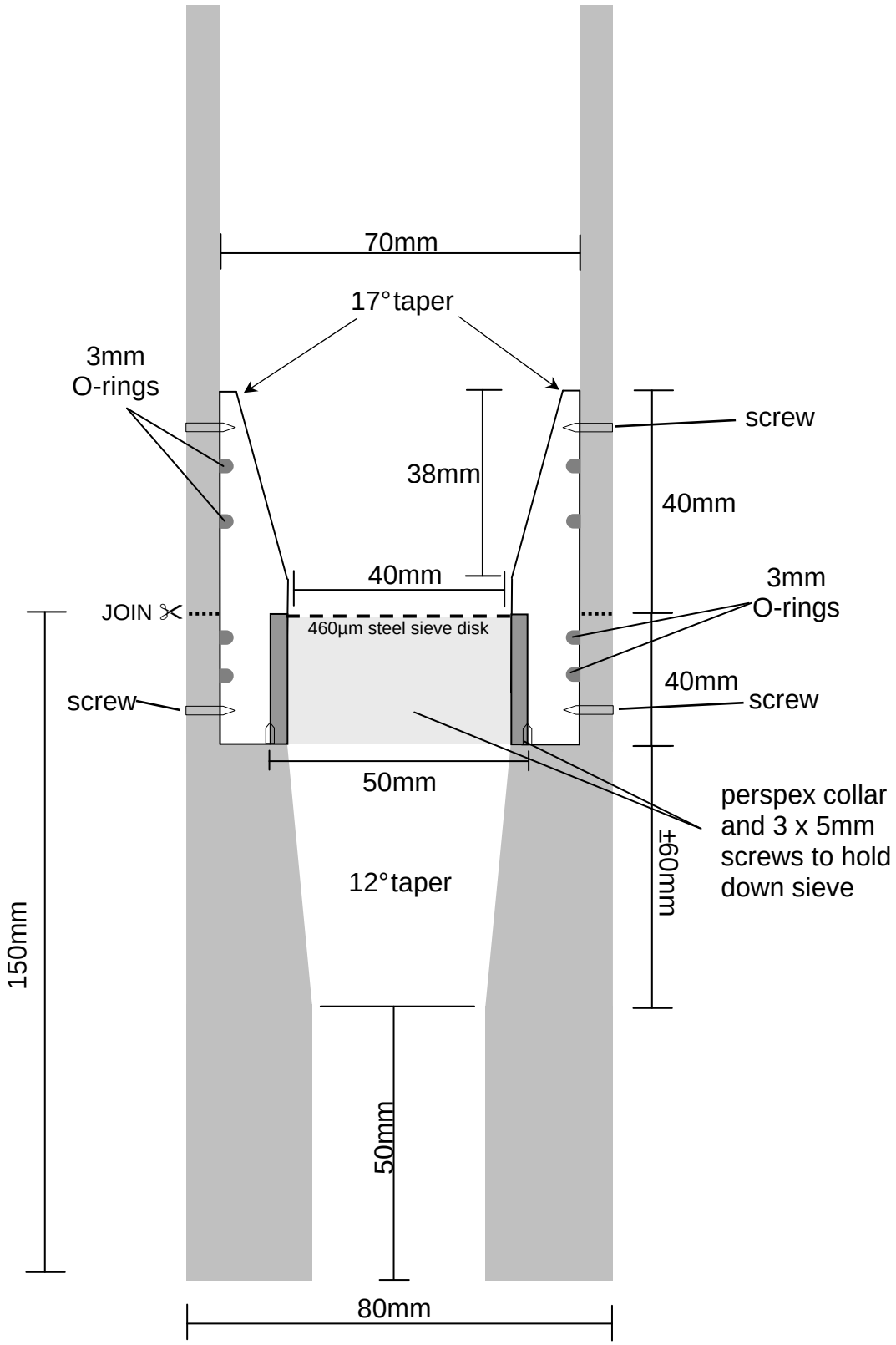

Figure c2: Dimensions of the conical diffuser portion of the fluidised bed reactor. The bottom section of the diffuser was machined out of a solid perspex rod. Double O-rings were included to prevent any escape of liquid from within the reactor.

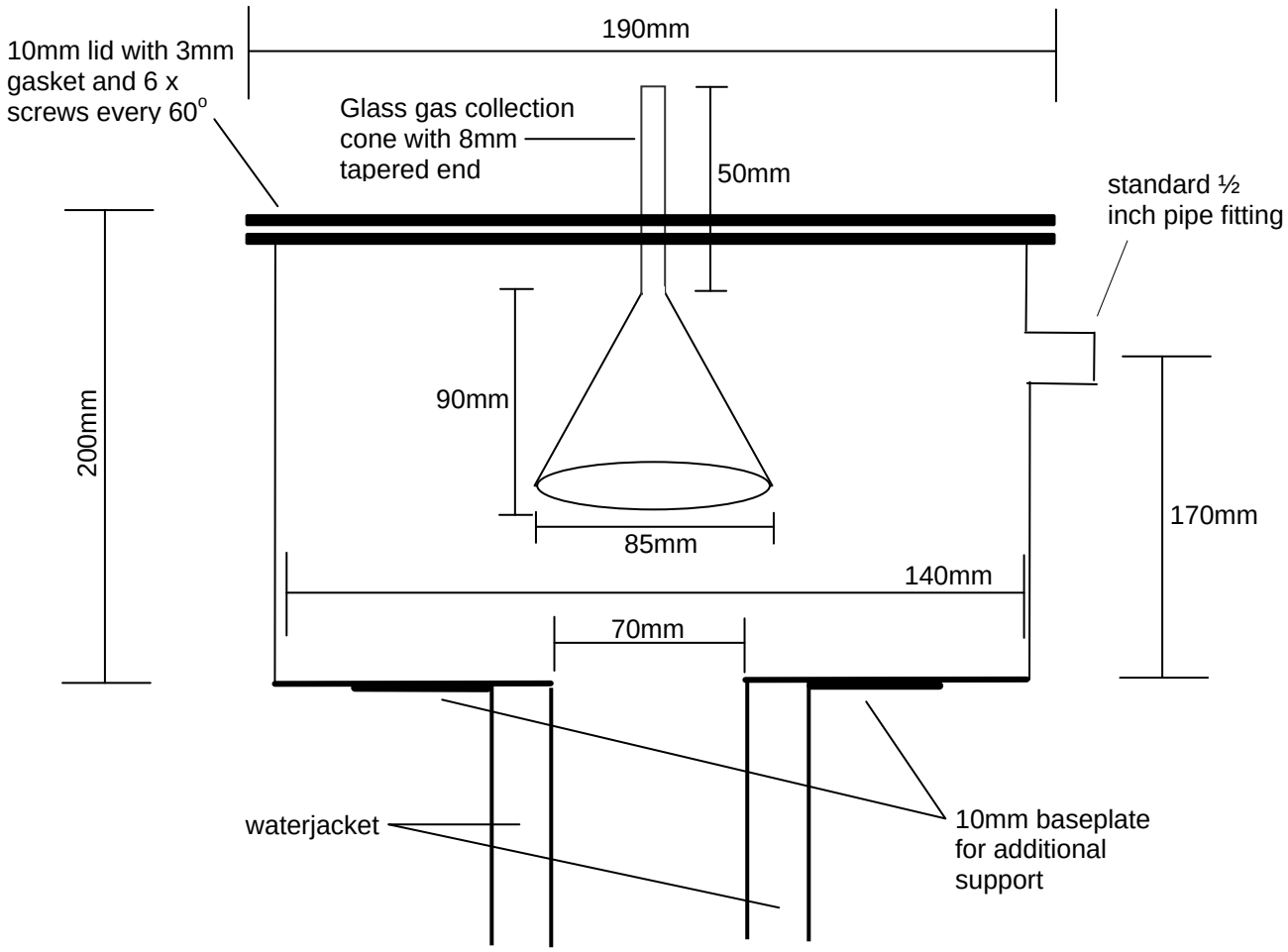

Figure c3: Technical schematic of the gas-solid separator portion of the fluidised bed. The glass cone is inserted and glued through the lid of the reactor. The glass cone is positioned so that it is able to catch all gas coming out of the central core of the reactor from the GAC bed.

Design of Acidified Water Gas Collector

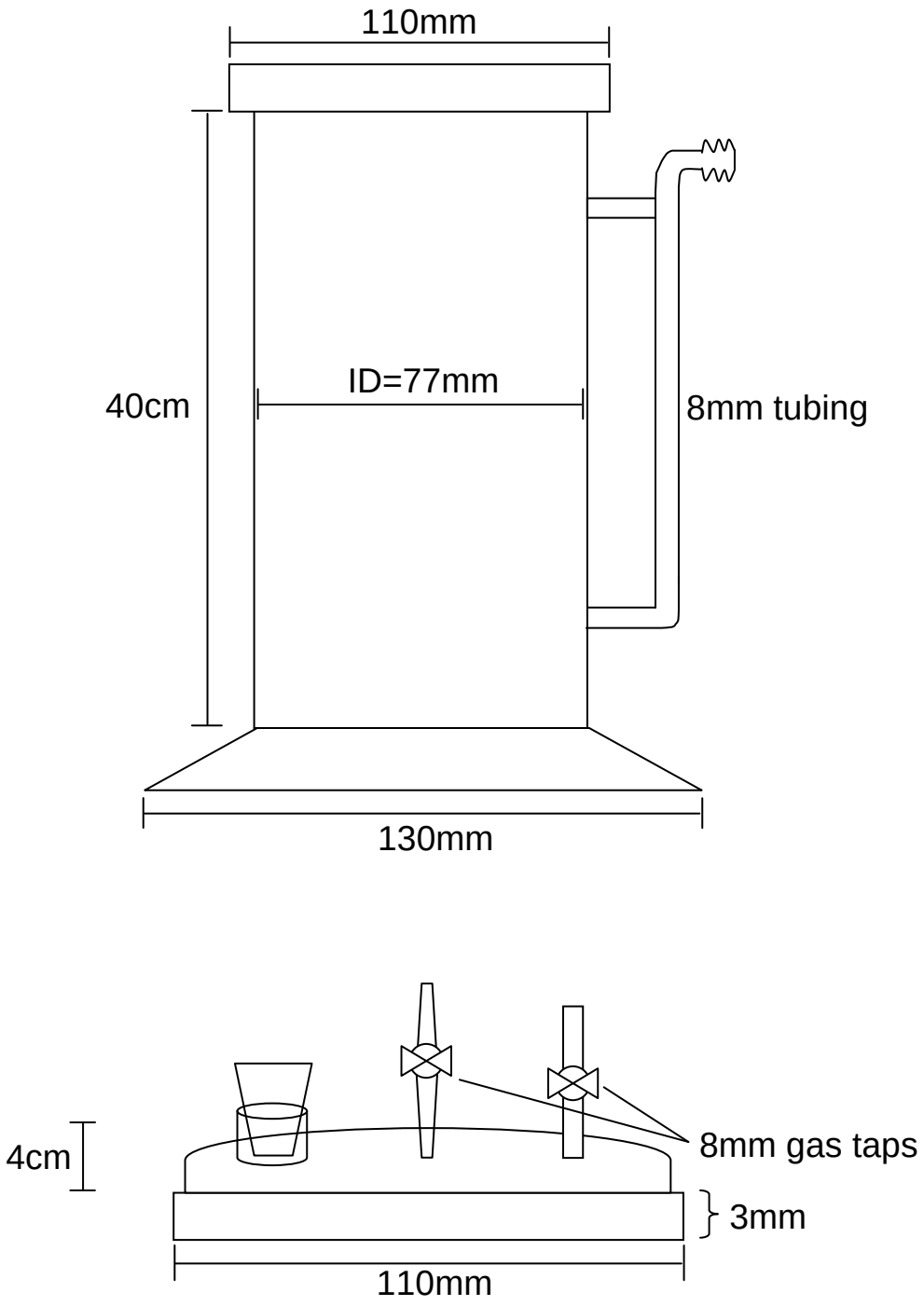

Figure c4: Technical schematic of portion A of the gas collector in which the acidified water from Standard Methods is placed (Franson, Clesceri *et al.* 1998). The internal diameter of the vessel is 77mm making every 2.18cm equal to 100ml based on the calculation of $(100cm^3)/\pi(3.8)^2$).

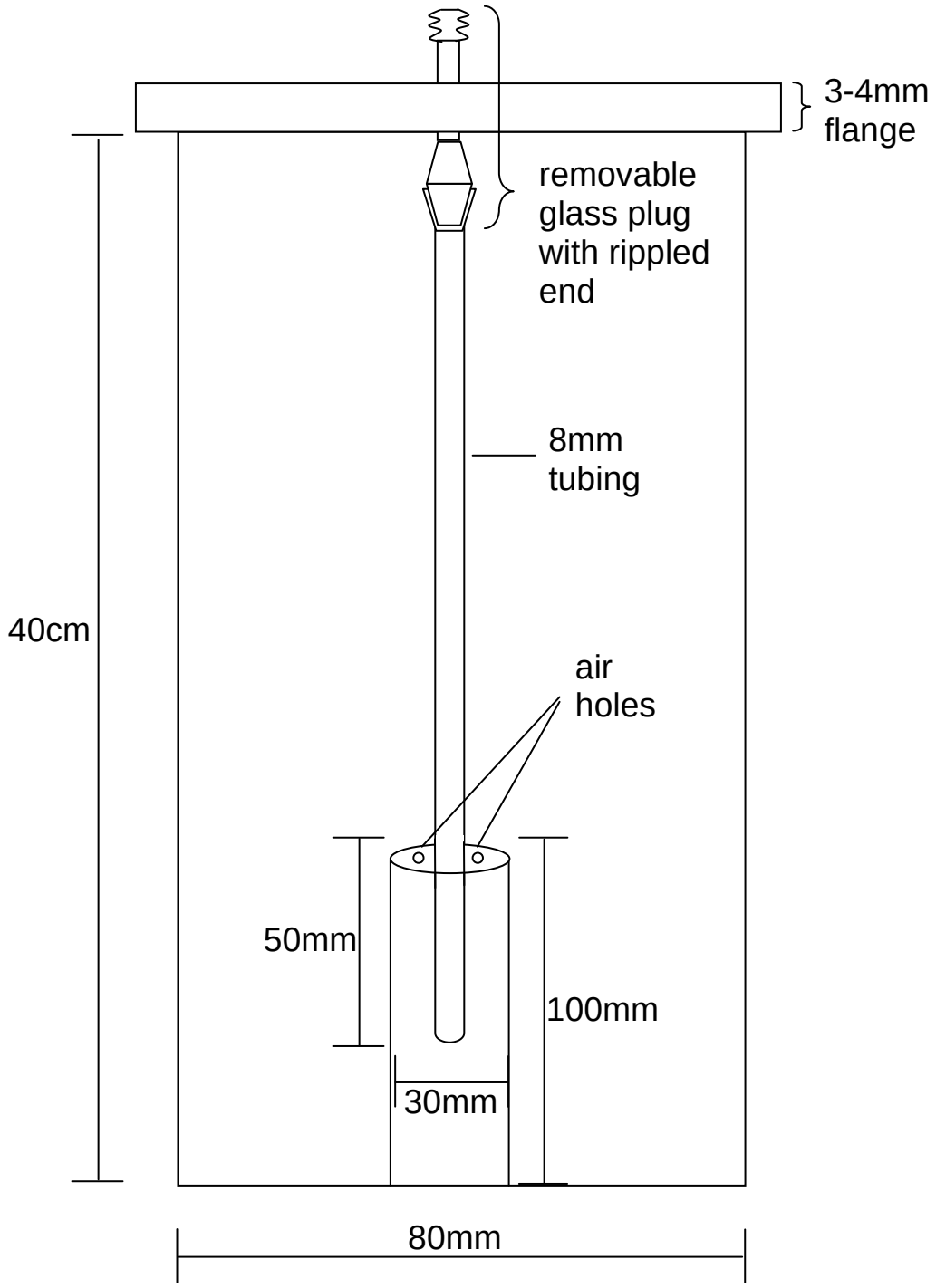

Figure c5: Technical schematic of portion B of the gas collector in which the acidified would flow into from portion A.

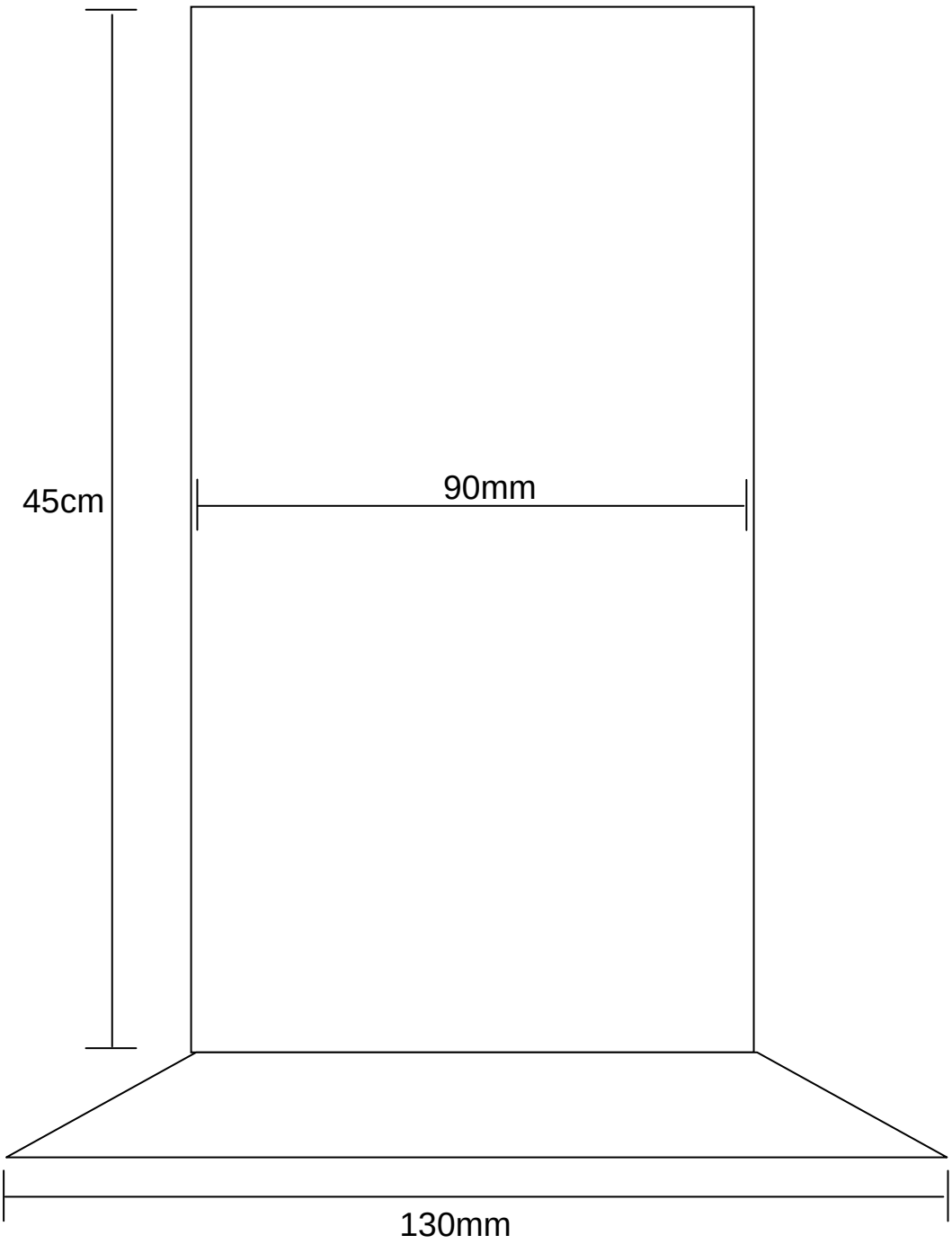

Figure c6: Technical schematic of portion C of the gas collector in which portion B would be placed while suspended on water. This portion is placed inside a bucket to collect the water that flows out of the top of the vessel.

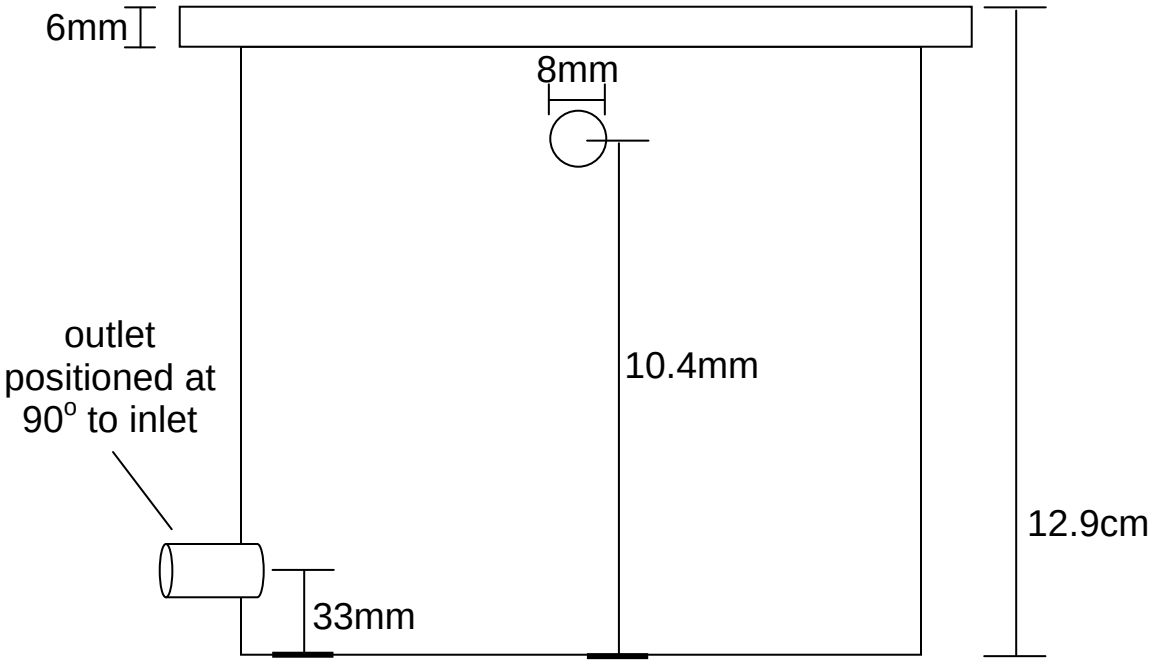

Figure c7: Dimensions of the electrode vessel used to house the monitoring electrodes.

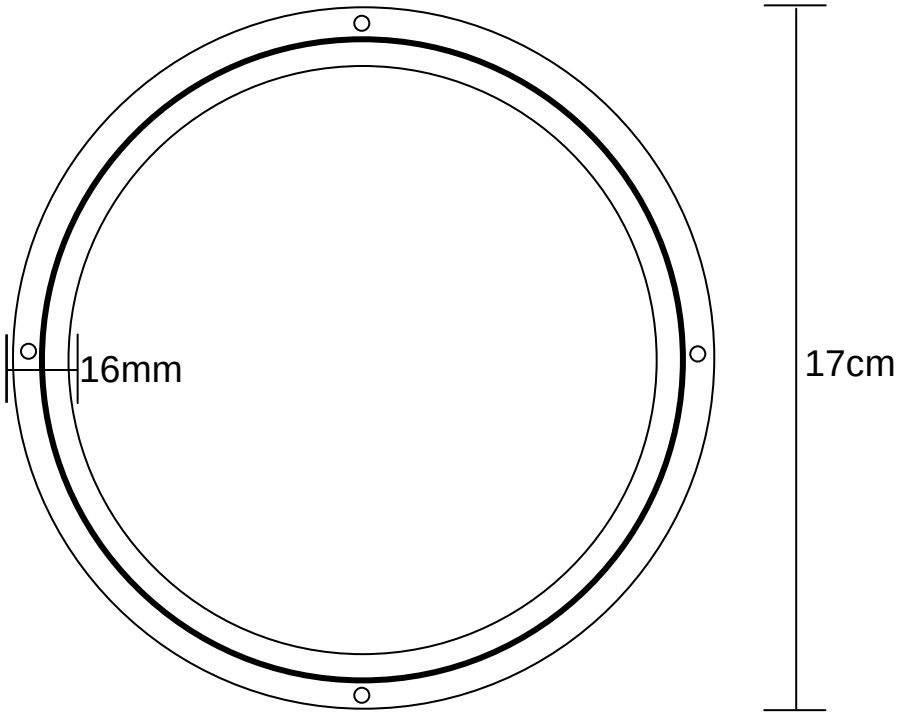

Figure c8: Top view of the electrode vessel used to house the electrodes.

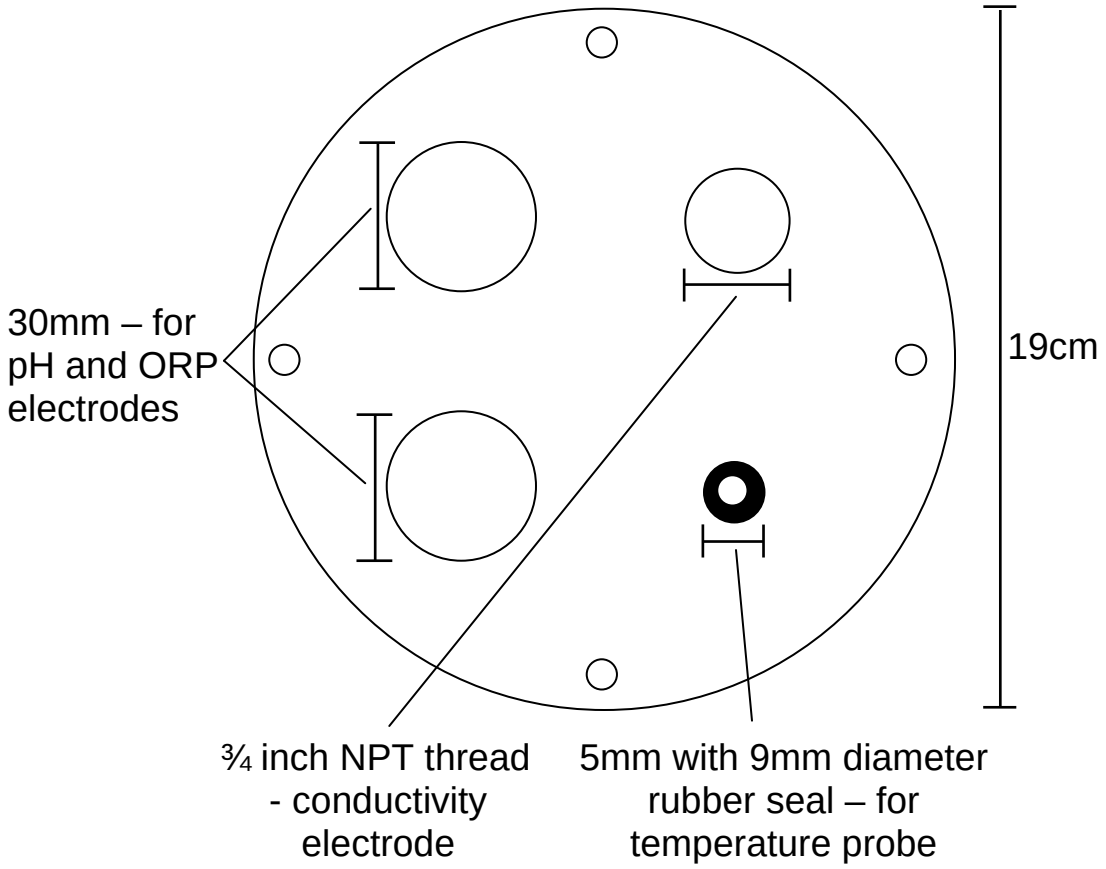

Figure c9: Lid of the electrode vessel into which the electrodes were fitted during the monitoring process.

REFERENCES

1. Allan, V. J. M., Callow M. E., Macaskie L. E. and Paterson-Beedle M. (2002). "Effect of nutrient limitation on biofilm formation and phosphatase activity of a *Citrobacter* sp." Microbiology **148**: 277-288.

2. Allison, D. G. (1998). "Exopolysaccharide production in bacterial biofilms." Biofilm Journal **3**(2).

3. Alves, C. F., Melo L. F. and Vieira M. J. (2002). "Influence of medium composition on the characteristics of a denitrifying biofilm formed by *Alcaligenes denitrificans* in a fluidised bed reactor." Process Biochemistry **37**: 837-845.

4. Ammary, B. Y. (2004). "Nutrients requirements in biological industrial wastewater treatment." African Journal of Biotechnology **3**(4): 236-238.

5. Andrews, G. F. (1982). "Fluidized Bed Fermenters: A Steady State analysis." Biotechnology and Bioengineering **24**: 2013-2030.

6. Angenent, L. T., Karim K., Al-Dahhan M. H., Wrenn B. A. and Domíguez-Espinosa R. (2004). "Production of bioenergy and biochemicals from industrial and agricultural wastewater." TRENDS in Biotechnology **22**(9): 477-485.

7. Archer, D. B., Hilton M. G., Adams P. and Wiecko H. (1986). "Hydrogen as a process control in a pilot scale anaerobic digester." Biotechnology Letters **8**(3): 197-202.

8. Asada, Y. and Miyake J. (1999). "Photobiological hydrogen production." Journal of Bioscience and Bioengineering **88**: 1-6.

9. Atlas, R. M. and Bartha R. (1998). Microbial Ecology - Fundamentals and Applications. USA, Benjamin/Cummins Publishing Company.

10. Baird, M. E. and Middleton J. H. (2004). "On relating physical limits to the carbon: nitrogen ratio of unicellular algae and benthic plants." Journal of Marine Systems **49**(1-4): 169-175.

11. Benemann, J. (1996). "Hydrogen Biotechnology: Progress and prospects." Nature Biotechnology **14**: 1101-1103.

12. Beynon, R. (1996). Making Buffers. http://www.bi.umist.ac.uk/users/mjfrbn/buffers/makebuf.asp. Last Viewed June 2004

13. Bockris, J. O. M. (2002). "The origin of ideas on a Hydrogen Economy and its solution to the decay of the environment." International Journal of Hydrogen Energy **27**: 731-740.

14. Boening, P. H. and Larsen V. F. (1982). "Anaerobic Fluidized Bed Whey Treatment." Biotechnology and Bioengineering **24**: 2539-2556.

15. Bosander, J. and Westlund Å. D. (2000). "Operation of full-scale fluidized bed for denitrification." Water Science and Technology **41**(9): 115-121.

16. Buffiére, P., Fonade C. and Moletta R. (1998). "Mixing and Phase Hold-Ups Variations Due to Gas Production in Anaerobic Fluidized-Bed Digesters: Influence on Reactor Performance." Biotechnology and Bioengineering **60**(1): 36-43.

17. Bull, M. A., Sterritt R. M. and Lester J. N. (1984). "An evaluation of single- and separated- phase anaerobic industrial wastewater treatment in Fluidized Bed Reactors." Biotechnology and Bioengineering **26**: 1054-1065.

18. Chang, F.-Y. and Lin C.-Y. (2004). "Biohydrogen production using an upflow anaerobic sludge blanket reactor." International Journal of Hydrogen Energy **29**: 33-39.

19. Chang, H. T., Rittman B.E., Amar D., Heim R., Ehrlinger O. and Lesty Y. (1991). "Biofilm detachment mechanisms in a liquid fluidized bed." Biotechnology and Bioengineering **38**: 499-506.

20. Chang, J.-S., Lee K.-S. and Lin P.-J. (2002). "Biohydrogen production with fixed-bed bioreactors." International Journal of Hydrogen Energy **27**: 1167-1174.

21. Chen, C. C. and Lin C. Y. (2001). "Start-up of Anaerobic Hydrogen Producing Reactors Seeded with Sewage Sludge." Acta Biotechnologica **21**(4): 371-379.

22. Chen, C.-C., Lin C.-Y. and Chang J.-S. (2001). "Kinetics of hydrogen production with continuous anaerobic cultures utilizing sucrose as the limiting substrate." Applied Microbiology and Biotechnology **57**: 56-64.

23. Chin, H.-L., Chen Z.-S. and Chou C. P. (2003). "Fedbatch Operation Using *Clostridium acetobutylicum* Suspension Culture as Biocatalyst for Enhancing Hydrogen Production." Biotechnology Progress **19**(2): 383-388.

24. Christensen, B. E. and Characklis W. G. (1990). Physical and Chemical Properties of Biofilms. New York, Wiley.

25. Coelhoso, I., Boaventura R. and Rodrigues A. (1992). "Biofilm reactors: An experimental and modeling study of wastewater denitrification in fluidised-bed bioreactors of activated carbon particles." Biotechnology and Bioengineering **40**: 625-633.

26. Cohen, A. (1982). Optimisation of anaerobic digestion of soluble carbohydrate-containing wastewaters by phase separation. Laboratorium voor Microbiologie, Universiteit van Amsterdam.

27. Cohen, A., Distel B., van Deursen A. and van Andel J. G. (1985). "Role of anaerobic spore-forming bacteria in the acidogenesis of glucose - changes induced by discontinuous or low-rate feed supply." Antonie van Leeuwenhoek Journal of Microbiology **51**(2): 179-192.

28. Cohen, A., Zoetemeyer R. J., van Deursen A. and van Andel J. G. (1979). "Anaerobic Digestion of Glucose with Separated Acid Production and Methane Formation." Water Research **13**: 571-580.

29. Cole, G. T. and Kendrick W. B. (1968). "Conidium ontogeny in hyphomycetes. The imperfect state of *Monascus ruber* and its meristem arthrospores." Canadian Journal of Botany **46**: 986-992.

30. Collet, C., Adler N., Schwitzguébel J.-P. and Péringer P. (2004). "Hydrogen production by *Clostridium thermolacticum* during continuous fermentation of lactose." International Journal of Hydrogen Energy **29**: 1479-1485.

31. Costa, C., Dijkema C., Friedrich M., García-Encina P., Fernández-Polanco F. and Stams A. J. M. (2000). "Denitrification with methane as electron donor in oxygen-limited bioreactors." Applied Microbiology and Biotechnology **53**: 754-762.

32. Costerton, J. W. and Lewandowski Z. (1995). "Microbial Biofilms." Annual Review in Microbiology **49**: 711-745.

33. Couderc, J.-P. (1985). Incipient Fluidization and Particulate Systems. Fluidisation. Harrison, D. London, Academic Press**:** 1-44.

34. Cowan, M. M., Warren T. M. and Fletcher M. (1991). "Mixed species colonization of solid surfaces in laboratory biofilms." Biofouling **3**: 23-34.

35. Dabrock, B., Bahl H. and Gottschalk G. (1992). "Parameters affecting solvent production by *Clostridium pasteurianum*." Applied and Environmental Microbiology **54**(4): 1233-1239.

36. Davey, M. E. and O'Toole G. A. (2000). "Microbial Biofilms: from Ecology to Molecular Genetics." Microbiology and Molecular Biology Reviews **64**(4): 847-867.

37. De Corte, B., Dries D., Verstraete W., Stevens P., Goossens L., De Vos P. and De Ley J. (1989). "The effect of the H_2 partial pressure on the metabolite pattern of *Lactobacillus casei, Escherichia coli* and *Clostridium butyricum*." Biotechnology Letters **11**(8): 583-588.

38. Denac, M. and Dunn I. J. (1988). "Packed- and Fluidized-Bed Biofilm Reactor Performance for Anaerobic Wastewater Treatment." Biotechnology and Bioengineering **32**: 159-173.

39. Dohanyos, M. (1985). "Production and inhibition of volatile fatty acids in various types of anaerobic reactors." Water Science and Technology **17**: 191-205.

40. Donlan, R. M. (2002). "Biofilms: Microbial Life on Surfaces." Emerging Infectious Diseases **8**(9): 881-890.

41. du Plessis, C. A., Senior E. and Hughes J. C. (1998). "Growth kinetics of microbial colonisation of porous media." South African Journal of Science **94**: 33-38.

42. Dunn, S. (2002). "Hydrogen futures: toward a sustainable energy system." International Journal of Hydrogen Energy **27**: 235-264.

43. El-Farhan, M. H. and Shieh W. K. (1999). "Overloading responses of a glucose-fed anaerobic fluidized bed reactor." Biochemical Engineering Journal **3**: 17-23.

44. Elmitwalli, T., Zeeman G. and Lettinga G. (2001). "Anaerobic treatment of domestic sewage at low temperature." Water Science and Technology **44**(4): 33-40.

45. Endo, G., Noike T. and Matsumoto J. (1982). "Characteristics of cellulose and glucose decomposition in acidogenic phase of anaerobic digestion (in Japanese)." Proceedings of the Society of Civil Engineering **325**: 61-68.

46. Eramo, B., Gavasci R., Misiti A. and Viotti P. (1994). "Validation of a multisubstrate mathematical model for the simulation of the denitrification process in fluidized bed biofilm reactors." Water Science and Technology **29**(10-11): 401-408.

47. Fang, H. H. P. and Liu H. (2002). "Effect of pH on hydrogen production from glucose by a mixed culture." Bioresource Technology **82**: 87-93.

48. Fang, H. H. P., Liu H. and Zhang T. (2002). "Characterization of a hydrogen-producing granular sludge." Biotechnology and Bioengineering **78**(1): 44-52.

49. Fox, P., Suidan M. T. and Bandy J. T. (1990). "A comparison of media types in acetate fed expanded bed anaerobic reactors." Water Research **24**(7): 827-835.

50. Franson, M. A. H., Clesceri L. S., Greenberg A. E. and Eaton A. D., Eds. (1998). Standard Methods for the Examination of Water and Wastewater. Baltimore, American Public Health Association.

51. Fröhlich, S., Lotz M., Larson B., Lübbert A., Schügerl K. and Seekamp M. (1991b). "Characterization of a Pilot Plant Airlift Tower Loop Bioreactor: III. Evaluation of Local Properties of the Dispersed Gas Phase during Yeast Cultivation and in a Model Media." Biotechnology and Bioengineering **38**: 56.

52. Garcia, J.-L., Patel B. K. C. and Ollivier B. (2000). "Taxonomic, Phylogenetic, and Ecological diversity of methanogenic *Archaea*." Anaerobe **6**: 205-226.

53. García-Calderón, D., Buffiére P., Moletta R. and Elmaleh S. (1998). "Influence of Biomass Accumulation on Bed Expansion Characteristics of a Down-Flow Anaerobic Fluidized-Bed Reactor." Biotechnology and Bioengineering **57**(2): 136-144.

54. Gaudy, A. and Gaudy E. (1981). Microbiology for Environmental Scientists and Engineers. Tokyo, McGraw-Hill Kogakusha, Ltd.

55. Gavrilescu, M. (2002). "Engineering concerns and new developments in anaerobic waste-water treatment." Clean Technology and Environmental Policy **3**: 346-362.

56. Georg Joergensen, R. and Scheu S. (1999). "Response of soil microorganisms to the addition of carbon, nitrogen and phosphorus in a forest Rendzina." Soil Biology and Biochemistry **31**(6): 859-866.

57. Gijzen, H. J. (2001). "Anaerobic digestion for sustainable development: a natural approach." Water Science and Technology **45**(10): 321-328.

58. Global Water Engineering Ltd. (2001). Worldwide experience. Anaerobic waste-water treatment. Last Viewed January 2004

59. Gray, C. T. and Gest H. (1965). "Biological formation of molecular hydrogen." Science **148**: 186-192.

60. Green, M., Ruskol Y., Lahav O. and Tarre S. (2001). "Chalk as the carrier for nitrifying biofilm in a fluidized bed reactor." Water Research **35**(1): 284-290.

61. Guwy, A. J., Hawkes F. R., Hawkes D. L. and Rozzi A. G. (1997). "Hydrogen Production in a High Rate Fluidised Bed Anaerobic Digester." Water Research **31**(6): 1291-1298.

62. Hallenbeck, P. C. and Benemann J. R. (2002). "Biological hydrogen production: Fundamentals and limiting processes." International Journal of Hydrogen Energy **27**: 1185-1193.

63. Harper, S. R. and Pohland F. G. (1986). "Recent developments in hydrogen management during anaerobic biological wastewater treatment." Biotechnology and Bioengineering **28**: 585-602.

64. Hashsham, S. A., Fernandez A. S., Dollhopf S. L., Dazzo F. B., Hickey R. F., Tiedje J. M. and Criddle C. S. (2000). "Parallel Processing of Substrate Correlates with Greater Functional Stability in Methanogenic Bioreactor Communities Perturbed by Glucose." Applied and Environmental Microbiology **66**(9): 4050-4057.

65. Hawkes, F. R., Dinsdale R., Hawkes D. L. and Hussy I. (2002). "Sustainable fermentative hydrogen production: challenges for process optimisation." International Journal of Hydrogen Energy **27**: 1339-1347.

66. Hawkes, F. R., Guwy A. J., Hawkes D. L. and Rozzi A. G. (1994). "On-line monitoring of anaerobic digestion: application of a device for continuous measurement of bicarbonate alkalinity." Water Science and Technology **30**(12): 1-10.

67. Heijnen, J. J., Mulder A., Enger W. and Hoeks F. (1989). "Review of the Application of Anaerobic Fluidized Bed Reactors in Waste-water Treatment." Chemical Engineering Journal **41**: B37-B50.

68. Hendrickx, T. L. G., Meskus E. and Keiski R. L. (2002). "Influence of the nutrient balance on biofilm composition in a fixed film process." Water Science and Technology **46**(4-5): 7-12.

69. Hidalgo, M. D. and García-Encina P. A. (2002). "Biofilm development and bed segregation in a methanogenic fluidized bed reactor." Water Research **36**: 3083-3091.

70. Holst, T. C., Truc A. and Pujol R. (1997). "Anaerobic Fluidized Beds: Ten Years of Industrial Experience." Water Science and Technology **36**(6-7): 415-422.

71. Hsu, Y. and Shieh W. K. (1993). "Startup of Anaerobic Fluidized Bed Reactors with Acetic Acid as the Substrate." Biotechnology and Bioengineering **41**: 347-353.

72. Hussy, I., Hawkes F. R., Dinsdale R. and Hawkes D. L. (2003). "Continuous fermentative hydrogen production from a wheat starch co-product by mixed microflora." Biotechnology and Bioengineering **84**(6): 619-626.

73. Inanc, B., Matsui S. and Ide S. (1996). "Propionic acid accumulation and controlling factors in anaerobic treatment of carbohydrate: Effects of H_2 and pH." Water Science and Technology **34**(5-6): 317-325.

74. Inanc, B., Matsui S. and Ide S. (1999). "Propionic acid accumulation in anaerobic digestion of carbohydrates: an investigation on the role of hydrogen gas." Water Science and Technology **40**(1): 93-100.

75. Janssen, A. J. H., Meijer S., Bontsema J. and Lettinga G. (1998). "Application of the Redox Potential for Controlling a Sulfide Oxidizing Bioreactor." Biotechnology and Bioengineering **60**(2): 147-155.

76. Jouanneau, Y., Lebecque S. and Vignais P. M. (1984). "Ammonia and light effect on nitrogenase activity in nitrogen-limited continuous cultures of *Rhodopseudomonas capsulata*: role of glutamate synthetase." International Journal of Hydrogen Energy **119**: 326-331.

77. Jung, G. Y., Kim J. R., Park J.-Y. and Park S. (2002). "Hydrogen production by a new chemoheterotrophic bacterium *Citrobacter* sp. Y19." International Journal of Hydrogen Energy **27**: 601-610.

78. Kalia, V. C., Jain S. R., Kumar A. and Joshi A. P. (1994). "Fermentation of biowaste of H_2 by *Bacillus licheniformis*." World Journal of Microbiology and Biotechnology **10**: 224-227.

79. Kalia, V. C., Lal S., Ghai R., Mandal M. and Chauhan A. (2003). "Mining genomic databases to identify novel hydrogen producers." TRENDS in Biotechnology **21**(4): 152-156.

80. Kanayama, H., Sode K. and Karube I. (1987). "Basic Studies of Hydrogen Evolution by *Escherichia coli* Containing a Cloned *Citrobacter freundii* Hydrogenase gene." Applied Biochemistry and Biotechnology **15**: 97-106.

81. Kerr, P. S., Huber S. C. and Israel D. W. (1984). "Effect of N-source on Soybean Leaf Sucrose Phosphate Synthase, Starch Formation, and Whole Plant Growth." Plant Physiology **75**: 483-488.

82. Khanal, S. K., Chen W.-H., Li L. and Sung S. (2004). "Biological hydrogen production: effects of pH and intermediate products." International Journal of Hydrogen Energy **29**: 1123-1131.

83. Khodadoust, A. P., Wagner J. A., Suidan M. T. and Brenner R. C. (1997). "Anaerobic Treatment of PCP in Fluidized-Bed GAC Bioreactors." Water Research **31**(7): 1776-1786.

84. Kim, I. S., Hwang M. H., Jang N. J., Hyun S. H. and Lee S. T. (2004). "Effect of low pH on the activity of hydrogen utilizing methanogen in bio-hydrogen process." International Journal of Hydrogen Energy **29**: 1133-1140.

85. Kjaergaard, L. (1977). The REDOX potential - its use and control in biotechnology. *Advances in Biochemical Engineering: Biotechnology*. Blakebrough, N. Berlin, Springer-Verlag. **7**.

86. Ko, I.-B. and Noike T. (2002). "Use of blue optical filters for suppression of growth of algae in hydrogen producing non-axenic cultures of *Rhodobacter sphaeroides* RV." International Journal of Hydrogen Energy(27): 1297-1302.

87. Kondo, T., Arawaka M., Wakayama T. and Miyake J. (2002). "Hydrogen production by combining two types of photosynthetic bacteria with different characteristics." International Journal of Hydrogen Energy(27): 1303-1308.

88. Koran, K. M., Suidan M. T., Khodadoust A. P., Sorial G. A. and Brenner R. C. (2001). "Effectiveness of an anaerobic granular activated fluidized-bed bioreactor to treat soil wash fluids: A proposed strategy for remediating PCP/PAH contaminated soils." Water Research **35**(10): 2363-2370.

89. Krieg, N. R. and Holt J. G., Eds. (1984). Bergey's Manual of Systematic Bacteriology. London, Williams and Wilkins.

90. Kumar, A., Jain S. R., Sharma C. B., Joshi A. P. and Kalia V. C. (1995). "Increased H_2 production by immobilized microorganisms." World Journal of Microbiology and Biotechnology **11**: 156-159.

91. Kumar, N. and Das D. (2001). "Electron microscopy of hydrogen producing immobilized *E. cloacae* IIT-BT 08 on natural polymers." International Journal of Hydrogen Energy **26**: 1155-1163.

92. Kumar, N., Monga P. S., Biswas A. K. and Das D. (2000). "Modeling and simulation of clean fuel production by *Enterobacter cloacae* IIT-BT 08." International Journal of Hydrogen Energy **25**: 945-952.

93. Lay, J.-J. (2000). "Modeling and Optimization of Anaerobic Digested Sludge Converting Starch to Hydrogen." Biotechnology and Bioengineering **68**(3): 270-278.

94. Lee, C.-Y., Huang J.-C. and Fellow A. S. C. E. (1995). "Novel Design for Constant-Pressure Water-Displacement Gas Collector." Journal of Environmental Engineering: 727-729.

95. Lee, Y. J., Miyahara T. and Noike T. (1999). Effect of pH on the microbial hydrogen fermentation. 6th International Association of Water Quality Asian-Pacific Conference, Taipei.

96. Lee, Y. J., Miyahara T. and Noike T. (2001). "Effect of Iron Concentration on Hydrogen Fermentation." Bioresource Technology **80**: 227-231.

97. Lengeler, J. W., Drews G. and Schlegel H. G. (1999). Biology of the Prokaryotes. Stuttgart, Blackwell Science.

98. Lettinga, G., van Velsen A. F. M., Hobma S. W., De Zeeuw W. and Klapwijk A. (1980). "Use of the upflow sludge blanket (USB) reactor concept for biological waste water treatment especially for anaerobic treatment." Biotechnology and Bioengineering **22**: 699-734.

99. Levin, D. B., Pitt L. and Love M. (2004). "Biohydrogen production: prospects and limitations to practical application." International Journal of Hydrogen Energy **29**: 173-185.

100. Li, B. and Bishop P. (2002). "Oxidation-reduction potential (ORP) regulation of nutrient removal in activated sludge wastewater treatment plants." Water Science and Technology **46**(1-2): 35-39.

101. Lin, C. Y. and Chang R. C. (1999). "Hydrogen production during the anaerobic acidogenic conversion of glucose." Journal of Chemical Technology and Biotechnology **74**: 498-500.

102. Lin, C. Y. and Lay C. H. (2004a). "Carbon/nitrogen-ratio effect on fermentative hydrogen production by mixed microflora." International Journal of Hydrogen Energy **29**: 41-45.

103. Lin, C. Y. and Lay C. H. (2005). "A nutrient formulation for fermentative hydrogen production using anaerobic sewage sludge microflora." International Journal of Hydrogen Energy **30**(3): 285-292.

104. Lin, C.-Y. and Lay C. H. (2004b). "Effects of carbonate and phosphate concentrations on hydrogen production using anaerobic sewage sludge microflora." International Journal of Hydrogen Energy **29**: 275-281.

105. Lindblad, P., Christensson K., Lindberg P., Federov A., Pinto F. and Tsygankov A. (2002). "Photoproduction of H_2 by wildtype *Anabaena* PCC 1720 and a hydrogen uptake deficient mutant: from laboratory to outdoor culture." International Journal of Hydrogen Energy(27): 1271-1281.

106. Lindsay, D. and von Holy A. (1997). "Evaluation of dislodging methods for laboratory grown bacterial biofilms." Food Microbiology **14**: 383-390.

107. Lindsay, D. and von Holy A. (1999). "Different responses of planktonic and attached *Bacillus subtilis* and *Pseudomonas fluorescens* to sanitizer treatment." Journal of Food Protection **62**: 368-379.

108. Linssen, J., Grube T., Hoehlein B. and Walbeck M. (2003). "Full fuel cycles and market potentials of future passenger car propulsion systems." International Journal of Hydrogen Energy **28**: 735-741.

109. Liu, Y. and Tay J.-H. (2002). "The essential role of hydrodynamic shear force in the formation of biofilm and granular sludge." Water Research **36**: 1653-1665.

110. Logan, B. E., Oh S.-E., Kim I. S. and Van Ginkel S. (2002). "Biological Hydrogen Production Measured in Batch Anaerobic Respirometers." Environmental Science and Technology **36**: 2530-2535.

111. Lopez, C., Pons M. N. and Morgenroth E. (2005). "Evaluation of microscopic techniques (epifluorescence microscopy, CLSM, TPE-LSM) as a basis for the quantitative image analysis of activated sludge." Water Research **39**(2-3): 456-468.

112. Maloney, S. W., Adrian N. R., Hickey R. F. and Heine R. L. (2002). "Anaerobic treatment of pinkwater in a fluidized bed reactor containing GAC." Journal of Hazardous Materials **92**: 77-88.

113. Marín, P., Alkalay D., Guerrero L., Chamy R. and Schiappacasse M. (1999). "Design and startup of an anaerobic fluidized bed reactor." Water Science and Technology **40**(8): 63-70.

114. Misra, C. and Gupta S. K. (2001). "Hybrid reactor for priority pollutant-trichloroethylene removal." Water Research **35**(1): 160-166.

115. Mizuno, O., Dinsdale R., Hawkes F. R., Hawkes D. L. and Noike T. (2000). "Enhancement of hydrogen production from glucose by nitrogen gas sparging." Bioresource Technology **73**(1): 59-65.

116. Moteleb, M. A., Suidan M. T., Kim J. and Maloney S. W. (2002). "Pertubated loading of a formaldehyde waste in an anaerobic granular activated carbon fluidized bed reactor." Water Research **36**: 3775-3785.

117. Mulcahy, L. T. and Shieh W. K. (1987). "Fluidization and reactor biomass characteristics of the denitrification fluidized bed biofilm reactor." Water Research **21**(4): 451-458.

118. Muslu, Y. (1987). "A new approach to the prediction of fluidisation of filter media." Water Research **21**(9): 1053-1060.

119. Nandi, R. and Sengupta S. (1998). "Microbial Production of Hydrogen: An Overview." Critical Reviews in Microbiology **24**(1): 61-84.

120. Ochieng, A., Odiyo J. O. and Mutsago M. (2003). "Biological treatment of mixed industrial wastewaters in a fluidised bed reactor." Journal of Hazardous Materials **B96**: 79-90.

121. Oh, S.-E., van Ginkel S. and Logan B. E. (2003). "The Relative Effectiveness of pH Control and Heat Treatment for Enhancing Biohydrogen Gas Production." Environmental Science and Technology **37**: 5186-5190.

122. Oh, Y.-K., Seol E.-H., Kim J. R. and Park S. (2003). "Fermentative biohydrogen production by a new chemoheterotrophic bacterium *Citrobacter* sp. Y19." International Journal of Hydrogen Energy **28**: 1353-1359.

123. Palazzi, E., Fabiano B. and Perego P. (2000). "Process development of continuous hydrogen production by *Enterobacter aerogenes* in a packed column reactor." Bioprocess Engineering **22**: 205-213.

124. Pauss, A. and Guiot S. R. (1993). "Hydrogen monitoring in anaerobic sludge bed reactors as various hydraulic retention times and loading rates." Water and Environmental Research **65**: 276-280.

125. Peyton, B. M. (1996). "Effects of shear stress and substrate loading rate on *Pseudomonas aeruginosa* biofilm thickness and density." Water Research **30**(1): 29-36.

126. Peyton, B. M. and Characklis W. G. (1993). "A statistical analysis of the effect of substrate utilization and shear stress on the kinetics of biofilm detachment." Biotechnology and Bioengineering **41**: 728-735.

127. Plisson-Saune, S., Capdeville B., Mauret M., Deguin A. and Baptiste P. (1996). "Real-Time Control of Nitrogen Removal Using Three ORP Bending-Points: Signification, Control Strategy and Results." Water Science and Technology **33**(1): 275-280.

128. Pratt, K. W., Koch W. F., Wu Y. C. and Berezansky P. A. (2001). "Molality-Based Primary Standards of Electrolytic Conductivity." Pure and Applied Chemistry **73**(11): 1783-1793.

129. Prescott, L. M., Harley J. P. and Klein D. A. (1999). Microbiology. United States of America, McGraw-Hill Companies Inc.

130. Pynaert, K., Smets B. F., Wyffels S., Beheydt D., Siciliano S. D. and Verstraete W. (2003). "Characterization of an Autotrophic Nitrogen-Removing Biofilm from a Highly Loaded Lab-Scale Rotating Biological Contactor." Applied and Environmental Microbiology **69**(6): 3626-3635.

131. Rachman, M. A., Furutani Y., Nakashimada Y., Kakizono T. and Nishio N. (1997). "Enhanced Hydrogen Production in Altered Mixed Acid Fermentation of Glucose by *Enterobacter aerogenes*." Journal of Fermentation and Bioengineering **83**(4): 358-363.

132. Rachman, M. A., Nakashimada Y., Kakizono T. and Nishio N. (1998). "Hydrogen production with high yield and high evolution rate by self-flocculated cells of *Enterobacter aerogenes* in a packed-bed reactor." Applied Microbiology and Biotechnology **49**: 450-454.

133. Ramachandran, R. and Menon R. K. (1998). "An Overview of the Industrial uses of Hydrogen." International Journal of Hydrogen Energy **23**(7): 593-598.

134. Ramsay, B. A., Wang D., Chavarie C., Rouleau D. and Ramsay J. A. (1991). "Penicillin Production in an Inverse Fluidized Bed Bioreactor." Journal of Fermentation and Bioengineering **72**: 495.

135. Ren, N., Wang B. and Huang J.-C. (1997). "Ethanol-Type Fermentation from Carbohydrate in High Rate Acidogenic Reactor." Biotechnology and Bioengineering **54**(5): 428-433.

136. Ren, N., Wang B. and Ma F. (1995). Hydrogen bio-production of carbohydrate fermentation by anaerobic sludge process. Proceedings of the 68th Annual Water Environmental Federal Conference, Miami.

137. Rovatti, M., Nicolella C., Converti A., Ghigliazza R. and Di Felice R. (1995). "Phosphorous removal in fluidized bed biological reactor (FBBR)." Water Research **29**(12): 2627-2634.

138. Rowan, N. J. and Anderson J. G. (1998). "Effects of above-optimum growth temperature and cell morphology on thermotolerance of *Listeria monocytogenes* cells suspended in bovine milk." Applied and Environmental Microbiology **64**: 2065-2071.

139. Rowan, N. J., Anderson J. G. and Candlish A. A. G. (2000). "Cellular morphology of rough forms of *Listeria monocytogenes* isolated from clinical and food samples." Letters in Applied Microbiology **31**: 319-322.

140. Saini, V. K., Bhandari S. C. and Tarafdar J. C. (2004). "Comparison of crop yield, soil microbial C, N and P, N-fixation, nodulation and mycorrhizal infection in inoculated and non-inoculated sorghum and chickpea crops." Field Crops Research **89**(1): 39-47.

141. Schink, B. (1997). "Energetics of syntrophic cooperation in methanogenic degradation." Microbiology and Molecular Biology Reviews **61**(2): 262-280.

142. Schnürer, A., Zellner G. and Svensson B. H. (1999). "Mesophilic syntrophic acetate oxidation during methane formation in biogas reactors." FEMS Microbiology Ecology **29**: 249-261.

143. Scott, C. D. and Hancher C. W. (1976). "Use of a Tapered Fluidized Bed as a Continuous Bioreactor." Biotechnology and Bioengineering **18**: 1393-1403.

144. Scott, D. S. (2004a). "Hydrogen - the case for inevitability." International Journal of Hydrogen Energy **29**: 227.

145. Scott, D. S. (2004b). "Okay! But Now Tell Me About Cost." International Journal of Hydrogen Energy **29**: 563-567.

146. Scott, D. S. (2005). "Fossil Sources: "Running Out" is *Not* the Problem." International Journal of Hydrogen Energy **30**: 1-7.

147. Sen, S. and Demirer G. N. (2003). "Anaerobic treatment of real textile wastewater with a fluidized bed reactor." Water Research **37**: 1868-1878.

148. Shen, B., Greenfield P. and Reid S. (1994). "Calcium Alginate Immobilized Hybridomas Grown Using a Fluidized-Bed Perfusion System with a Protein-Free Medium." Cytotechnology **14**: 109.

149. Shieh, W. K. and Hsu Y. (1996). "Biomass Loss from an Anaerobic Fluidized Bed Reactor." Water Research **30**(5): 1253-1257.

150. Shieh, W. K. and Keenan J. D. (1986). Fluidized Bed Biofilm Reactor for Wastewater Treatment. Advances in Biochemical Engineering/Biotechnology. Fiechter, A. Berlin, Springer-Verlag. **33:** 132-167.

151. Shin, H.-S., Youn J.-H. and Kim S.-H. (2004). "Hydrogen production from food waste in anaerobic mesophilic and thermophilic acidogenesis." International Journal of Hydrogen Energy **29**: 1355-1363.

152. Shreiner, R. H. (2002). "Stability of Standard Electrolytic Conductivity Solutions in Glass Containers." Journal of Research of the National Institute of Standards and Technology **107**(5): 393-399.

153. Sich, H. and van Rijn J. (1997). "Scanning electron microscopy of biofilm formation in denitrifying, fluidised bed bioreactors." Water Research **31**(4): 733-742.

154. Sipma, J., Lens P. N. L., Stams A. J. M. and Lettinga G. (2003). "Carbon monoxide conversion by anaerobic bioreactor sludges." FEMS Microbiology Ecology **44**: 271-277.

155. Spagni, A., Buday J., Ratini P. and Bortone G. (2001). "Experimental considerations on monitoring ORP, pH, conductivity and dissolved oxygen in nitrogen and phosphorus biological removal processes." Water Science and Technology **43**(11): 197-204.

156. Sreekrishnan, T. R., Ramachandran K. B. and Ghosh P. (1991). "Effect of operating variables on biofilm formation and performance of an anaerobic fluidized-bed bioreactor." Biotechnology and Bioengineering **37**: 557-566.

157. Steyer, J. P., Bouvier J. C., Conte T., Gras P., Harmand J. and Delgenes J. P. (2001). "On-line measurements of COD, TOC, VFA, total and partial alkalinity in anaerobic digestion processes using infra-red spectrometry." Water Science and Technology **45**(10): 133-138.

158. Steyer, J.-P., Genovesi A. and Harmand J. (2001). "Advanced monitoring and control of anaerobic wastewater treatment plants: fault detection and isolation." Water Science and Technology **43**(7): 183-190.

159. Straker, C. (2004). Personal Communication, University of the Witwatersrand.

160. Stronach, S. M., Diaz-Baez M. C., Rudd T. and Lester J. N. (1987). "Factors Affecting Biomass Attachment during Startup and Operation of Anaerobic Fluidized Beds." Biotechnology and Bioengineering **30**: 611-620.

161. Stronach, S. M., Rudd T. and Lester J. N. (1986). Anaerobic Digestion Processes in Industrial Wastewater Treatment - Biotechnology Monographs. Germany, Springer-Verlag.

162. Strong, G. E. and Cord-Ruwisch R. (1995). "An *In Situ* Dissolved-Hydrogen Probe for Monitoring Anaerobic Digesters Under Overload Conditions." Biotechnology and Bioengineering **45**: 63-68.

163. Suh, I. S., Schumpe A. and Deckwer W.-D. (1992). "Xanthan Production in Bubble Column and Air-Lift Reactors." Biotechnology and Bioengineering **39**: 85.

164. Suidan, M. T., Flora J. R. V., Boyer T. K., Wuellner A. M. and Narayanan B. (1996). "Anaerobic Dechlorination using a Fluidized-Bed GAC Reactor." Water Research **30**(1): 160-170.

165. Sun, Y. and Furusaki S. (1990). "Continuous Production of Acetic Acid using Immobilized *Acetobacter aceti* in a Three-Phase Fluidized Bed Bioreactor." Journal of Fermentation and Bioengineering **69**: 102.

166. Sutherland, I. W. (2001). "Biofilm exopolysaccharides: a strong and sticky framework." Microbiology **147**: 3-9.

167. Taguchi, F., Mizukami N., Yamada K., Hasegawa K. and Saito-Taki T. (1995). "Direct conversion of cellulosic materials to hydrogen by *Clostridium* sp. strain no. 2." Enzyme and Microbial Technology **17**: 147-150.

168. Tanisho, S. and Ishiwata Y. (1995). "Continuous Hydrogen Production from Molasses by Fermentation Using Urethane Foam as a Support of Flocks." International Journal of Hydrogen Energy **20**(7): 541-545.

169. Tanisho, S., Kuromoto M. and Kadokura N. (1998). "Effect of CO_2 removal on hydrogen production by fermentation." International Journal of Hydrogen Energy **23**(7): 559-563.

170. Terashima, S., Kamihira M., Ogawa T., Ohno M., Iijima S. and Kobayashi T. (1994). "Continuous Production of Human Erythropoietin by Immobilized Recombinant L-929 Cells." Journal of Fermentation and Bioengineering **77**: 52.

171. Thauer, R. K. (1990). "Energy metabolism of methanogenic bacteria." Biochimica et Biophysica Acta **1018**: 256-259.

172. To, M. S., Favrin S., Romanova N. and Griffiths M. W. (2002). "Postadaptational Resistance to Benzalkonium Chloride and Subsequent Physicochemical Modifications of *Listeria monocytogenes*." Applied and Environmental Microbiology **68**(11): 5258-5264.

173. Totzke, D. E. (2004). 2004 Anaerobic Treatment Technology Overview. Brookfield, Applied Technologies, Inc.**:** 1-13.

174. Ueno, Y., Haruta S., Ishii M. and Igarashi Y. (2001a). "Characterization of a microorganism isolated from the effluent of hydrogen fermentation by microflora." Journal of Bioscience and Bioengineering **92**(4): 397-400.

175. Ueno, Y., Haruta S., Ishii M. and Igarashi Y. (2001b). "Microbial community in anaerobic hydrogen-producing microflora enriched from sludge compost." Applied Microbiology and Biotechnology **57**: 555-562.

176. Ueno, Y., Otsuka S., , and Morimoto M. (1996). "Hydrogen Production from Industrial Wastewater by Anaerobic Microflora in Chemostat Culture." Journal of Fermentation and Bioengineering **82**(2): 194-197.

177. van Ginkel, S., Sung S. and Lay J.-J. (2001). "Biohydrogen Production as a Function of pH and Substrate Concentration." Environmental Science and Technology **35**: 4726-4730.

178. Vavilin, V. A., Lokshina L. Y., Rytov S. V., Kotsyurbenko O. R., Nozhevnikova A. N. and Parshina S. N. (1997). "Modeling methanogenesis during anaerobic conversion of complex organic matter at low temperatures." Water Science and Technology **36**(6-7): 531-538.

179. Vavilin, V. A., Rytow S. V. and Lokshina L. Y. (1995). "Modelling Hydrogen Partial Pressure Change as a Result of Competition between the Butyric and Propionic Groups of Acidogenic Bacteria." Bioresource Technology(54): 171-177.

180. Veziroglu, T. N. (1998). Hydrogen Energy System: A Permanent Solution to Global Problems. http://www.iahe.org. Last Viewed December 2004

181. von Uexküll, O. (2004). "Energy and Water - The Ignored Link." Refocus **MarchApril**: 40-44.

182. Webb, O. F., Davison B. H., Scott T. C. and Scott C. D. (1995). "Design and Demonstration of an Immobilized-Cell Fluidized-Bed Reactor for the Efficient Production of Ethanol." Appl. Biochem. Biotechnol. **51/52**: 559.

183. Wilson, G. J., Khodadoust A. P., Suidan M. T., Brenner R. C. and Acheson C. M. (1998). "Anaerobic/aerobic biodegradation of pentachlorophenol using GAC fluidized bed reactors: optimization of the empty bed contact time." Water Science and Technology **38**(7): 9-17.

184. Wu, J.-H. and Lin C.-Y. (2004). "Biohydrogen production by mesophilic fermentation of food wastewater." Water Science and Technology **49**(5-6): 223-228.

185. Wu, S.-Y., Lin C.-N. and Chang J.-S. (2003). "Hydrogen Production with Immobilized Sewage Sludge in Three-Phase Fluidized-Bed Bioreactors." Biotechnology Progress **19**: 828-832.

186. Wu, S.-Y., Lin C.-N., Chang J.-S., Lee K.-S. and Lin P.-J. (2002). "Microbial Hydrogen Production with Immobilized Sewage Sludge." Biotechnology Progress **18**(5): 921-926.

187. Wu, W.-M., Jani M. K., Conway de Macario E., Thiele J. H. and Zeikus J. G. (1992). "Microbial composition and characterisation of prevalent methanogens and acetogens isolated from syntrophic methanogenic granules." Applied Microbiology and Biotechnology **38**: 282-296.

188. Xiaojian, Z., Zhansheng W. and Xiasheng G. (1991). "Simple combination of biodegradation and carbon adsorption - the mechanism of the biological activated carbon process." Water Research **25**(2): 165-172.

189. Yokoi, H., Saitsu A., Uchida H., Hirose J., Hayashi S. and Takasaki Y. (2001). "Microbial Hydrogen Production from Sweet Potato Starch Residue." Journal of Bioscience and Bioengineering **91**(1): 58-63.

190. Yokoi, H., Tokushige T., Hirose J., Hayashi S. and Takasaki Y. (1998). "H_2 Production from Starch by a mixed culture of *Clostridium butyricum* and *Enterobacter aerogenes*." Biotechnology Letters **20**(2): 143-147.

191. Yu, H., Zhu Z., Hu W. and Zhang H. (2002). "Hydrogen production from rice winery wastewater in an upflow anaerobic reactor using mixed anaerobic cultures." International Journal of Hydrogen Energy **27**: 1359-1365.

192. Yu, H. Q. and Fang H. H. P. (2001). "Acidification of mid- and high strength dairy wastewater." Water Research **35**(5): 3697-3705.

193. Yu, R.-F., Liaw S.-L., Chang C.-N., Lu H.-J. and Cheng W.-Y. (1997). "Monitoring and Control Using On-line ORP on the Continuous-Flow Activated Sludge Batch Reactor System." Water Science and Technology **35**(1): 57-66.

194. Zhao, X., Hickey R. F. and Voice T. C. (1999). "Long-term evaluation of adsorption capacity in a biological activated carbon fluidized bed reactor system." Water Research **33**(13): 2983-2991.

195. Zhu, H., Ueda S., Asada Y. and Miyake J. (2002). "Hydrogen production as a novel process of wastewater treatment - studies on tofu wastewater with entrapped *R. sphaeroides* and mutagenesis." International Journal of Hydrogen Energy **27**: 1349-1357.

196. Zoetemeyer, R. J., Arnoldy P., Cohen A. and Boelhouwer C. (1982). "Influence of Temperature on the Anaerobic Acidification of Glucose in a Mixed Culture Forming Part of a Two-Stage Digestion Process." Water Research **16**: 313-321.

197. Zoetemeyer, R. J., Matthijsen A. J. C. M., Cohen A. and Boelhouwer C. (1982). "Product Inhibition in the Acid Forming Stage of the Anaerobic Digestion Process." Water Research **16**: 633-639.

198. Zoetemeyer, R. J., Van Den Heuvel J. L. and Cohen A. (1982). "pH influence on acidogenic dissimilation of glucose in an anaerobic digester." Water Research **16**: 303-311.