

The Role of Microorganisms in The Nutrient Improvement of Urea Fermented Brewer's Dried Grains

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ABSTRACT

The role of microorganisms in the improvement of the nutritive value of urea fermented brewer's dried grains (BDG) was investigated. One kilogramme of BDG was weighed into two containers each and thoroughly mixed with 1% and 2% urea solutions, put into two thick black polythene bags, sealed and stored under shade to ferment for 7 days. Wet samples of the 1% and 2% urea fermented BDG were used for microbial identification and population count by microscopy and culture test methods. Proximate and detergent fibre compositions showed significant ($P < 0.05$) improvement in the crude protein content of urea fermented BDG. The crude fibre contents were significantly ($P < 0.05$) decreased with increased urea concentration. The fibre fractions of urea fermented BDG were significantly ($P < 0.05$) reduced. Bacteria organisms were identified and found to be responsible for fibre breakdown in urea fermented BDG. Improved crude protein contents resulted from fibre breakdown by bacteria and the bacteria proteins.

Keywords: Urea, Fermentation, Concentration, Brewer's dried grains, Nutritive value, Microorganisms

1.0 INTRODUCTION

The use of agro industrial by-products and farm waste has been advocated and many of these by-products have been used in practical poultry diets. The problem of availability and cost of by-products have made the drive or their use as feed ingredients a more complicated issue. Any by-product not directly required by man and which can be obtained readily at a very low price with a good combination of nutrients is the best desired. Brewer's dried grains (BDG) a by-product of the brewery industry has an amino acid profile similar to that of groundnut cake, Table 1 (Aduku, 1993; Atteh *et al.*, 2000). Although the protein content of brewer's dried grain (24.21 to 27.90%) is lower than that of groundnut cake (45.00%), the difference is a quantitative issue (Aduku, 1993; Isikwenu *et al.*, 2008). It means the problem of substituting the more expensive groundnut cake (GNC) protein with the cheaper BDG protein is a quantitative problem. Meanwhile the level of incorporation of BDG into practical poultry diets has been limited by its high proportion of crude fibre content (Isikwenu *et al.*, 2008; Onwudike, 1993; Ademosun, 1973; Richardson *et al.*, 1958). To up-grade BDG's nutritive value, the crude fibre content will have to be reduced by fibre breakdown. Several chemical and biological methods for up grading fibrous materials have been developed (IFRU, 2003). Faniyi *et al.*, (1997 and 1999) successfully used urea fermentation and biodegradation in poultry faeces to significantly improve the crude protein contents and significantly reduce the crude fibre fractions in cowpea and sorghum seedhulls. This observation is supported by other reports on alkali treatment which causes extensive delignification and improved feed value (Isikwenu, 2006; Willis *et al.*, 1980) caused significant improvement in feed: gain and net energy values (Isikwenu *et al.*, 2008; Garret *et al.*, 1979), increased rate and efficiency of gain (Isikwenu *et al.*, 2010; Saxena *et al.*, 1971) and good animal performance (Klopfestein, 1978). However, the role of microorganisms that may be involved in the chemical and biodegradation processes for up-grading fibrous materials has not been investigated. This study was conducted to investigate the involvement of microorganisms in crude fibre breakdown in BDG through urea fermentation of BDG.

2.0 MATERIALS AND METHODS

The brewer's dried grains used was derived entirely from maize and sorghum grains and sourced from Guinness Plc Factory, Benin City, Nigeria.

2.1 Preparation of urea solution

Urea made of 46% N (fertilizer grade) was used to prepare 1% and 2% concentrations of urea solutions. To obtain 2% urea solution containing 20g urea per litre of water, 400g of urea was dissolved in 20 litres of clean water while 1% urea solution was obtained by dissolving 200g of urea in 20 litres of clean water with the solution containing 10g urea per litre of water.

2.2 Urea fermentation of brewer's dried grains

One kilogramme of BDG was each weighed into two containers and one litre of the urea solutions (1% and 2% respectively) were added to each container. The BDG and urea solutions were thoroughly mixed to obtain uniform wetness and the excess urea solution was allowed to drain off by placing the mixtures on standard sieves. The mixtures were then put into two thick black polythene bags, properly sealed and stored under shade

to ferment for 7 days. At the end of the fermentation period, the products were sun dried and preserved for chemical analysis of proximate compositions.

2.3 Microbial identification and population count.

At the end of the fermentation period, wet samples of the 1% and 2% urea fermented BDG were taken for microbial identification and population count of the microorganisms involved in the fermentation process, using the microscopic and culture test methods. One gram of the wet samples from each urea solutions were emulsified in a drop of normal saline on a microscopic slide and covered with a cover slip. The samples were then examined with X10 objective lens and the organisms found were identified with X40 objective lens. One gram of the wet samples from each urea solutions were also inoculated into peptone water and incubated for 8 hours at 37°C. The solutions were then sub-cultured with a wire loop into Desoxycholate Citrate Agar (DCA) and Maconkey plates and incubated at 37°C for 24 hours. Isolates from culture were biochemically identified using lactose, indole, ureases, oxidase, citrate and coagulase reagents.

2.4 Chemical analysis

The homogenized samples of urea fermented BDG and the untreated BDG were analyzed for their proximate composition by the procedures of AOAC (1990) and detergent fibre fractions by the method of Goering and Van Soest (1975).

2.5 Statistical analysis

Data obtained were subjected to analysis of variance and treatment means were compared by Duncan's Multiple Range Test (Duncan, 1955) using SPSS 10.0 package.

3.0 RESULTS

The proximate compositions of the urea fermented BDG and the untreated BDG are presented in Table 2. The results of the chemical analysis revealed that urea fermented BDG had significant improvement in crude protein, ether extract, and crude fibre contents over the untreated BDG. The crude protein, ether extract and crude fibre values of 35.50%, 4.73% and 4.59% for 1% urea solution and 38.52%, 4.87% and 4.49% for 2% urea solutions of urea fermented BDG were significantly ($P < 0.05$) better than that of the untreated BDG which had 24.21%, 3.69% and 11.20% of crude protein, ether extract and crude fibre respectively. The ash, organic matter and gross energy values were similar ($P > 0.05$) for all samples. However, the nitrogen free extract (NFE) of untreated BDG was significantly ($P < 0.05$) higher than that of 2% concentration of urea fermented BDG but similar ($P > 0.05$) to that of 1% urea concentration. The detergent fibre analysis results of urea fermented and untreated BDG are presented in Table 3. The results of the detergent fibre analysis of urea fermented and untreated BDG showed a significant ($P < 0.05$) reduction in values of all the fibre fractions. The neutral detergent fibre (NDF), acid detergent fibre (ADF), acid detergent lignin (ADL), cellulose and hemicelluloses contents of the untreated BDG were significantly ($P < 0.05$) higher than those of all urea fermented BDG.

The results of the biochemical identification reagents reactions are presented in Table 4. The mixed growth colonies from agar culture media consists of two major groups namely: Mucoid yellowish colonies and spreading pink colonies with fishly odour. The mucoid yellowish colonies were identified as predominantly *Escherichia coli* and reacted positively to indole and lactose tests. The spreading pink colonies with fishly odour were identified as predominantly *Proteus vulgaris* and reacted positively to indole, urease and citrate tests. Other microorganisms present were not significant to grow on the agar media. The results of the microbial (bacteria) load from serial dilution of the cultured substrates are presented in Table 5. The approximate numbers of bacteria colony-forming unit per ml for 1% urea cultured substrate was CFU = 25,000 CFU/ml and that of 2% urea cultured substrate was CFU = 30,000 CFU/ml. The fermented 2% urea solution sample had more bacterial colonies than the fermented 1% urea solution sample. From the tests carried out, fermentation activities was higher in the substrate with 2% urea than that of 1% urea as obtained from visual observation, colour, odour and moisture content. The substrate with 2% urea concentration had more moisture content, darker colour and a stronger odour compared with that of 1% urea concentration.

4.0 DISCUSSION

The result of the chemical analysis of the untreated BDG used in this study are 24.12% crude protein, 11.20% crude fibre, 3.69% ether extract and 8.04% ash and these results are in agreement with those obtained by Oluyemi *et al.* (1979), 23.30% crude protein, 17.50% crude fibre, 4.10% ether extract and 6.00% ash; Aduku (1993), 27.90% crude protein, 11.70% crude fibre, 7.40% ether extract and 4.80% ash and Uchegbu and Udedibie (1998), 28.25% crude protein, 13.12% crude fibre, 6.79% ether extract and 7.36% ash. Savory and gentle (1976) reported that fibre consists of cellulose which is a carbohydrate but difficult to digest and utilize.

Hughes (1991) and Akinsoyinu (1999) have reported that dietary fibre resist enzymatic digestion in the gastrointestinal tract of monogastric animals. The resistance to enzymatic digestion by fibre in monogastric is caused by the lignin component which forms a complex with cellulose and hemicelluloses. It also contains some phenolic compounds such as ferulic and diferulic acids bonded to it by ester linkages between the carboxylic groups of the acids and the hydroxyl groups of the polysaccharides, especially hemicelluloses (Hartley, 1981). Therefore, to make the nutrients in fibre available components for digestion in monogastrics, the cellwall components need to be ruptured so that the cellulose and hemicelluloses can be released. It is expected that since fibre consists of soluble and insoluble portions, attempt to predigest BDG by urea fermentation would make nutrients in them available for use by monogastrics. The urea fermentation of BDG effected positive changes in its chemical composition and hence changes in nutritive value. The crude protein content of the 1% and 2% urea fermented BDG increased significantly and this result is supported by the findings of Hadjipanayiotou *et al.* (1993), Brown (1994), McDonald *et al.* (1995), Faniyi *et al.* (1997 a & b, 1999) and Isikwenu (2006) who obtained increased crude protein contents in fibrous materials treated with alkali and fermented or not fermented. The increase in crude protein could be due to the breakdown of the structural components of fibre in BDG by the degradation of the ester bonds or linkages in their fibre (McDonald *et al.*, 1995). It is possible that there was a weakening of the binding forces in lignin due to the reactions with urea and as such encouraged the breakdown of fibre into simpler substances. This would have facilitated the solubilization of hemicellulose even if they did not change the cellulose content (Klopfestein, 1978). The reactions initiated a process of disintegration which was further enhanced by fermentation in urea solution. This probably explains the significantly reduced crude fibre content of the urea fermented BDG when compared to the untreated BDG. The significant reduction of the crude fibre content and detergent fibre fractions in the urea fermented BDG showed that the use of urea is effective in improving the nutritive value of BDG for use in poultry feeds. The disintegration of the ligno-cellulose complex, hemicellulose-cellulose bonds and other physical structural components by the hydrolytic reactions would have caused a released of locked up nutrients such as lipids, sugar, soluble proteins, organic acid, non-protein nitrogen and other water soluble substances. The degradation of the detergent fibre fractions resulted in increased nutritive value of BDG as shown in the proximate composition and detergent fibre fractions of urea fermented BDG in Tables 2 and 3. The results from this study agree with the findings of Mayhard *et al.* (1979), McDonald *et al.* (1995), Faniyi *et al.* (1997a and 1999), Lewis *et al.* (1999), Vipond *et al.* (2001), Bui *et al.* (2001), Chesson (2001) and Isikwenu (2006) who obtained improved nutritive value from various fibrous materials of farm residues and agro-industrial by-products when treated with urea or other alkali substances. The soluble carbohydrates (NFE) content reduced with urea fermentation. It implies that the reaction initiated in fibre breakdown with urea fermentation requires the use of energy which would have been readily supplied by the available soluble carbohydrates in the fermented BDG. The slight increase in gross energy values was due to increased release of lock-up lipids in the cell walls and thus compensated for used energy since lipids contain more energy per unit than carbohydrate, agreeing with results obtained by Abou-Raya *et al.* (1971), Garret *et al.* (1979) and Willis *et al.* (1980).

The two major bacteria organisms that were identified and isolated were *E. coli* and *Proteus vulgaris*, although other microorganisms were present but they were not significant to grow on the agar media. The addition of urea to the substrate increased the activity of the bacteria organisms during the fermentation process. This is because bacteria break down urea to obtain its nitrogen that is essential for increasing the growth of the bacteria organisms by multiplying the organisms at a fast rate. This agrees with Jurgens (1979) who stated that bacteria will combine nitrogen from dietary protein or non-protein nitrogen sources with a carbon skeleton from carbohydrate sources to form their own body proteins. The substrate with 2% urea concentration was found to have higher bacteria population (30,000 CFU/ml) than the one with 1% urea concentration (25,000 CFU/ml). This shows that the higher urea concentration provided more available nitrogen for increased growth rate and rapid multiplication of bacteria organisms in the 2% urea fermented BDG. The microbial origin of urease (an enzyme) which is involved in the breakdown of urea to release nitrogen have been substantiated by other workers (Allison, 1969, McDonald *et al.*, 1995). Jurgens (1979) have reported that bacteria contain enzymes that rupture cellulose, hemicelluloses and starch bonds or linkages in the breakdown of carbohydrate for the release of volatile fatty acids (VFA). It therefore, follows that the presence of bacteria organisms in the 1% and 2% urea fermented BDG will produce fibre breakdown during fermentation. The higher the population of bacteria, the more of their enzymes that will be available for the breakdown of the crude fibre in the urea fermented BDG. The 2% urea concentration fermented BDG with higher bacteria population had more breakdown of crude fibre as shown by their proximate composition and fibre fractions in Tables 2 and 3. The increased crude protein contents of the urea fermented BDG may not have resulted from the release of lock-up nutrients only but also from the conversion of the non-protein nitrogen from urea to form the body protein of bacteria involved in the fibre breakdown process. These bacteria organisms will significantly enhance the protein content and quality of the urea fermented dried brewer's grain.

5.0 CONCLUSION

The degradation of the crude fibre content, the significant increase in crude protein content and the slightly moderate increase in ether extract and gross energy are indicative of the fact that urea fermentation of BDG can enhance its nutritional value as a feed ingredient. The bacteria identification and population count indicate that microorganisms were responsible for fibre breakdown in the urea fermented BDG and that higher urea concentration means higher microbial presence and more fibre breakdown in the fermentation process.

6.0 REFERENCES

- Abou-Raya, A. K., Abou El-Hassan, A and Al-Rahad, P. A. (1971). The Feeding value of peanut hay and its response to alkali treatment and addition of urea. *U.A.R.J. Animal Prod.* 11:309.
- Ademosun, A. A. (1973). Evaluation of Brewer's dried grains in diets of growing chickens. *Brit. Poul. Sci* 14:463-468.
- Aduku, A. O. (1993). *Tropical Feedstuff Analysis Table*. Ahmadu Bello University, Samaru-Zaria, Nigeria.
- Akinsoyinu, A. O. (1999). *Chemistry and Nutritional Value of Nigeria Feed and feeding stuff*. University of Ibadan, Ibadan, Nigeria.
- Allison, M. J. (1969). Nitrogen metabolism of ruminant microorganisms. In *Physiology of Digestion and Metabolism in the Ruminant*. Proc. of 3rd Int. Symp. Cambridge, England, August, 1969, 456-473.
- Association of Official Analytical Chemists (A O. A. C., 1990). *Official methods of Analysis*, 15th edition, Washington D.C.
- Atteh, J. O. and Opawande, F. E. (2000). Replacement of brewer's dried grain for groundnut cake in broiler finisher diets. Proc. of 25th Annual Conf. of Nigeria Society for Animal Production (NSAP), 19th-23rd March, 2000. Michael Okpara University of Agriculture, Umudike. 165-168.
- Brown, W. F. (1994). Urea Treatment: A New Hay Ammoniation Method. *The Florida Cattleman and Livestock Jour.* (1994).
- Bui Van Chinh, L. E., Viet, L. Y., Ngugyen Hiju Tao, Nguyen Van Hai and Tran Bich Ngoc. (2001). Effect of drying, ensiling or urea-treatment on the use of sugarcane leaves as ruminant feed. Proc. Workshop on improved utilization of By-products for animal feeding in Vietnam-NUFU Project 3/2001.
- Chesson, A. (2001). Non-starch polysaccharide degrading enzymes in poultry diets: Influence of ingredients on selection of activities. *World Poul. Sci. Jour.* 57:251-263
- Duncan, D. B. (1955). Multiple Range and multiple F-tests. *Biometric* 11:1-42
- Faniyi, G. E. and Ologhobo, A. D. (1999). Effects of replacing brewer's dried grains with lye and urea treated cowpea and sorghum seedhulls in broiler diets. *Trop. Anim. Prod. Invest.* 2:69-82.
- Faniyi, G. E. Ologhobo, A. D. Adeniran, G A and Alaka, O. O. (1997a). Chemical characterization of untreated and treated cowpea and sorghum seedhulls used as poultry feed ingredients: In: *Livestock Products*. Proc. 2nd Ann. Conf. Anim. Sci. Assoc. of Nigeria, Sept 16th – 17th, 1997, Ikeja, Lagos. 41-52.
- Faniyi, G.E. Ologhobo, A. D., Adeniran, G.A. and Alaka, O. O. (1997b). Protein enrichment of cowpea and sorghum seedhulls for poultry feeding by fermentation in poultry faeces. In: *Livestock Products*. Proc. 2nd Ann. Conf. Anim. Sci. Assoc. of Nigeria, Sept 16th – 17th, 1997, Ikeja, Lagos 52-63.
- Garret, W. N., Walker, H. G., Kohler, G. O. and Hart, M. R. (1979). Response of ruminants to diets containing sodium hydroxide or ammonia treated rice straw. *Jour. Anim. Sci.* 48(1):92-102.
- Goering, H. K. and Van Soest, P. J. (1975). Forage fibre analysis. *Agr. Handbook No. 379*, A.R.S., U.S.D.A
- Hadjipanayiotou, M., Verhaeghe, L., Goodchild, T. and Shaker, B. (1993). Ammonization of straw using urea, ammonia gas or ammonium hydroxide. *Livestock Res. Rural Dev.* (5) 3:1-5.
- Hartley, R. A. (1981). In *Agricultural Science Seminar*. Degradation of plant cell materials. 15-21. Agric. Res. Council, London.
- Hughes, J. S. (1991). Potential Contribution of dry bean dietary fibre to health. *Food Technology*. 122-126.
- International Feed Resources Unit (IFRU, 2003) Roughages as a ruminant feed: Evaluation of nutritive value of roughages. The Macaulay Land Use Research Institute, Aberdeen, UK.
- Isikwenu, J. O. (2006). The use of urea-treated and fermented brewer's dried grains in poultry diets. Ph.D Thesis, Department of Animal Science, Delta State University, Abraka.
- Isikwenu, J. O. Omeje, S. I., Okagbare, G. O. and Akpodiete, O. J. (2008). Effects of replacing groundnut cake with urea-fermented brewer's dried grains in broiler chicks diets. *Anim. Res. Int.* 5(1):795-800.
- Isikwenu, J. O., Akpodiete, O. J. Omeje, S. I. and Okagbare, G. O. (2010). The effects of replacing groundnut cake with urea-treated and fermented brewer's dried grains on nutrient digestibility, retention and carcass characteristics of broiler finishers. *Nig. Jour, Anim. Prod.* 37(1):1-12.

- Jurgens, M. H. (1979). *Animal Feeding and Nutrition*. 4th ed. Kendall/Hunt Publishing Company, 2460 Kerper Boulevard, Dubuque, Iowa 52001. 33.
- Klopfestein, T. (1978). Chemical treatment of crop residues. *Jour. Anim. Sci* 46(3): 841-848.
- Lewis, M. I., Lowman, B. D. and Ford, M. (1999). Urea treatment as a means of preserving/processing moist wheat for intensively finished cattle. *Proc. of Brit. Soc. Anim. Sci.* 54.
- Maynard, L. A., Loosli, J. K. Hintz, H. F. and Warner, R. G. (1979). *Animal Nutrition. Carbohydrates and their metabolism*. *Anim Feed Sci. and Tech.* 21:99-125.
- McDonald, P., Edwards, R. A. and Greenhalgh, I. F.D. (1995). *Animal Nutrition*. 5th ed. Longman Scientific and Technical, England.
- Oluyemi, J. A. and Roberts, F. A. (1979). *Poultry production in warm wet climates*. 1st ed. Macmillan Press Limited, London.
- Onwudike, O. C. (1993). Energy and protein requirements of broiler chicks in the humid tropics. *Trop. Anim. Prod. Invest.* 8:39-44.
- Richardson, C. E., Watts, A. B. and Epps, E. A. (1958). The effect of added fibre with or without fat in practical broiler ration. *Poult. Sci.* 37:1278.
- Savory, C. J. and Gentle, K. J. (1976). Effects of dietary dilution with fibre on the food intake and gut dimensions of Japanese Quail. *Brit. Poult. Sci.* 17:560-570.
- Saxena, S. K., Otterby, D. E., Donker, J. D. and Good, A. L. (1971). Effects of feeding alkali-treated straw supplemented with soyabean meal or non-protein nitrogen on growth of lambs and on certain blood and rumen liquor parameters. *J. Anim. Sci.* 33:485-487.
- Statistical Package for Social Sciences (SPSS). Version 10.0. SPSS Inc. Chicago, IL, 60606.
- Uchegbu, M. C. and Udedibie, A. B. I. (1998). Maize/Sorghum-based dried brewer's grains in broiler finisher diets. *Nig. Jour. Anim. Prod.* 25(1 & 2): 13-16.
- Vipond, J. E., Lewis, M and Allcroft, D. T. (2001). Urea preserved grain for finishing lambs. *Proc. Brit. Soc. Anim. Sci.* (2001).
- Willis, C. M., Stallcup, O. T. and Kreider, D. L. (1980). Influence of sodium hydroxide and enzyme additions on nutritive value of rice straw. *Jour. Anim. Sci.* 50(2): 303-310.

Table 1: Chemical Composition of Groundnut Cake and Brewer's Dried Grains

Parameters	GNC	Untreated BDG
Crude protein	45.00	27.90
Ether extract	9.16	7.40
Crude fibre	3.81	11.70
Ash	5.51	4.80
Calcium	0.20	0.30
Phosphorous	0.60	0.88
Lysine	1.73	0.90
Methionime	0.44	0.60
Cystein	0.72	0.40
Arginine	5.00	1.30
Tryptophan	0.49	0.40
ME Kcal/kg (Swine)	3185.00	2240.00
ME Kcal/kg (Poultry)	2530.00	2513.00

Source: Aduku (1993)

Table 2: Proximate Composition of Urea Fermented and Untreated BDG

Parameters (%)	Untreated BDG	Urea fermented BDG (1% Conc.)	Urea fermented BDG (2% Conc.)
Dry matter	93.34 ± 0.42	89.12 ± 0.87	88.76 ± 2.31
Crude protein	24.21 ± 1.16 ^b	35.57 ± 3.60 ^a	38.52 ± 7.26 ^a
Crude fibre	11.20 ± 1.65 ^b	4.59 ± 0.31 ^a	4.49 ± 0.03 ^a
Ether extract	3.69 ± 0.89	4.73 ± 1.11	4.87 ± 0.33
Ash	8.04 ± 0.50	6.05 ± 2.24	5.99 ± 2.24
NFE	46.20 ± 2.23 ^a	38.18 ± 5.69 ^{ab}	34.89 ± 10.67 ^b
Organic Matter	85.30 ± 1.10	83.07 ± 0.32	82.77 ± 0.03
Calculated: Gross Energy (Kcal/g)	5.14 ± 0.18	5.33 ± 1.01	5.17 ± 0.64

a, b means with different superscripts in the same row are significantly (P < 0.05) different

BDG: Brewer's dried grains

Table 3: Detergent Fibre Analysis of Urea Fermented and Untreated BDG

Parameters (%)	Untreated BDG	Urea fermented BDG (1% Conc.)	Urea fermented BDG (2% Conc.)
Neutral Detergent Fibre (NDF)	66.11 ± 0.15 ^a	56.12 ± 0.11 ^b	50.10 ± 0.55 ^c
Acid Detergent Fibre (ADF)	52.79 ± 0.32 ^a	46.72 ± 0.83 ^b	42.98 ± 0.76 ^c
Acid Detergent Lignin (ADL)	0.61 ± 0.02 ^a	0.41 ± 0.02 ^b	0.28 ± 0.01 ^c
Cellulose	52.18 ± 0.09 ^a	46.31 ± 0.44 ^b	42.43 ± 0.29 ^c
Hemicellulose	13.11 ± 0.54 ^a	9.40 ± 0.38 ^b	7.12 ± 0.17 ^c

a, b, c means with different superscripts in the same row are significantly (P < 0.01) different

BDG: Brewer's dried grains

Table 4: Biochemical Identification Reagents Reactions

Mixed growth colonies from Agar culture Media	Reagents						Organism identified
	Indole	Urease	Oxidase	Citrate	Coagulase	Lactose	
Mucoid Yellowish colonies	+	-	-	-	-	+	<i>E. Coli</i>
Spreading Pink colonies with fishly odour	+	+	-	+	-	-	<i>Proteus vulgaris</i>

Table 5: Bacterial Population Count (Numbers of bacteria colonies)

Substrate Serial Dilution	Culture media	No. of Bacteria colony counted	Approximate no of bacteria colony-forming unit per ml – 1/100
1% Urea Substrate 1/80	Maconkey Agar Media	20	$CFU = \frac{1}{50} \div 500$ $CFU = 50 \times 500$ = 25,000 bacteria per ml
2% Urea Substrate 1/80	Maconkey Agar Media	30	$CFU = \frac{1}{60} \div 500$ $CFU = 60 \times 500$ = 30,000 bacteria per ml