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P173 Atlântico room

178

Fehling's method for quantifying total reducing sugars in hydrolyzed cellulose pulp

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INTRODUCTION

The woods are mainly composed of cellulose, hemicellulose and lignin. To convert cellulose to ethanol two main steps are involved: breaking the chains of cellulose molecules into sugars and fermenting those sugars into ethanol. To determine the potential formation of reducing sugars in cellulose pulp, two steps are required: the acid hydrolysis and the measurement of the concentration of these sugars. Optimization of acid hydrolysis of cellulose pulp will allow reducing sugars from cellulose with the lowest possible cost.

The aim of this study is to test an adapted methodology to evaluate the reducing sugars formation from hydrolyzed cellulose, to use as a model for future studies with wood biomass. Chromatographic methods are expensive and complexes and others simple analysis (as DNS and phenol sulfuric acid) use toxic reagents. The Fehling's method needs adjustments. This method is validated in two situations: a) on standard glucose solutions, b) in analysis of the hydrolysate of cellulose pulp.

RESULTS AND DISCUSSION

In order to validate the method with solutions of glucose is necessary to add 5 mL of Fehling A, 5 mL of Fehling B and the sample according the Table 1.

Warm the mixture and extend boiling for two more minutes. Cool the solution to 15°C. Add 1.5 mL of potassium iodide 30%, 5 ml of sulfuric acid 17%. Titrate with sodium thiosulfate 0.1 N, using as indicator 1 mL of starch 1%. The content of total reducing sugars expressed as g/L which corresponds to the difference between the required volume to titrate the sample and the volume titration of the blank, provided in Table 2.

With different concentrations of glucose solutions (0.5 to 10 g/L) is possible to verify a low variation coefficient between measurements of quadruplicate (highest value=9.4%, concentration=0.5 g/L). The equation is: $[glucose]_{Fehling} = 1.07\,[glucose] - 0.15$; R^2 0.9965.

Table 1. Sample and distilled water volumes to determine the sugar concentration.[1]

Range of concentration of sugars (g/L)	Volume of sample (mL)	Volume of distilled water (mL)	Multiplication factor
9,0	5	5	1
18,0	2,5	7,5	2
45,0	1	9	5
90,0	0,5	9,5	10

The acid hydrolysis of cellulose pulp is accomplished according to reference 2. Hydrolysis results indicate that this process provided an average yield of 90.9% in relation to the theoretical maximum calculated (range 1.3 to 5.2 g/L of glucose). The adapted method has good results for the concentration range tested, where R^2 has a value of 0.9868.

CONCLUSION

This Fehling analysis for total reducing sugars proved to be adequate to quantify glucose concentrations in cellulose pulp after acid hydrolysis, in screening tests.

ACKNOWLEDGEMENTS

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REFERENCES

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Table 2. Correspondence between the solution volume of solution of sodium thiosulfate 0.1 N and the amount of reducing sugars in g/L, where V_{spent} = volume consumed in the blank titration - volume consumed in the sample titration, in mL.[1]

V_{spent}	0,0	0,05	0,1	0,15	0,2	0,25	0,3	0,35	0,4	0,45	0,5	0,55	0,6	0,65	0,7	0,75	0,8	0,85	0,9	0,95
0	0,00	0,04	0,08	0,12	0,16	0,20	0,24	0,27	0,31	0,34	0,38	0,41	0,44	0,48	0,51	0,54	0,58	0,61	0,65	0,68
1	0,72	0,75	0,78	0,81	0,85	0,88	0,92	0,95	0,98	1,02	1,05	1,09	1,12	1,15	1,19	1,22	1,26	1,29	1,32	1,36
2	1,39	1,43	1,46	1,49	1,53	1,56	1,60	1,63	1,66	1,70	1,73	1,76	1,79	1,83	1,87	1,90	1,93	1,96	2,00	2,03
3	2,06	2,10	2,14	2,17	2,21	2,24	2,27	2,31	2,34	2,37	2,40	2,44	2,48	2,51	2,54	2,57	2,61	2,65	2,68	2,71
4	2,74	2,78	2,81	2,84	2,88	2,91	2,95	2,99	3,02	3,05	3,08	3,12	3,16	3,19	3,23	3,26	3,30	3,34	3,37	3,41
5	3,44	3,47	3,51	3,54	3,58	3,62	3,65	3,69	3,72	3,76	3,80	3,83	3,87	3,90	3,93	3,97	4,00	4,04	4,07	4,11
6	4,15	4,18	4,22	4,25	4,29	4,32	4,35	4,39	4,42	4,46	4,50	4,53	4,57	4,60	4,64	4,68	4,71	4,75	4,78	4,81
7	4,85	4,88	4,92	4,96	4,99	5,03	5,06	5,10	5,14	5,17	5,21	5,25	5,29	5,33	5,36	5,40	5,43	5,47	5,51	5,54
8	5,58	5,61	5,65	5,69	5,72	5,76	5,79	5,83	5,87	5,91	5,95	5,98	6,02	6,06	6,09	6,13	6,16	6,20	6,24	6,27
9	6,31	6,34	6,38	6,42	6,45	6,49	6,53	6,57	6,61	6,64	6,68	6,72	6,75	6,79	6,82	6,86	6,90	6,93	6,97	7,00
10	7,04	7,08	7,11	7,15	7,19	7,23	7,27	7,30	7,34	7,37	7,41	7,45	7,48	7,52	7,56	7,60	7,64	7,68	7,72	7,76
11	7,80	7,84	7,88	7,92	7,95	7,99	8,03	8,07	8,11	8,15	8,19	8,23	8,27	8,31	8,35	8,39	8,43	8,47	8,51	8,55
12	8,59	8,63	8,66	8,70	8,74	8,78	8,82	8,86	8,90	8,94	8,98	9,01	9,05	9,09	9,12	9,16	9,19	9,23	9,27	9,31