Evaluation Of Bacterial Profile And Proximate Analysis Of Corn Waste

[1]Obasi, Nnenna Patrick., [2]Ifeanyi, Virginia O. and [3]Ayogu, Chukwuemerie Victor.
[1]Aquatic Bioresources Training Centre, Adiabo, Cross River State, National Biotechnology Development Agency.
[2]Department of Microbiology, Michael Okpara University of Agriculture, Umudike, Abia State.
Corresponding author: chikagoodness@gmail.com: 08032108485

Abstract: Corn waste contains moisture, cellulose, hemicellulose and lignin which make it a good raw material for making substrate in the production of organic acids with the aid of bacteria. These bacteria, during metabolism are able to produce acids that can be used as solvents. The proximate analysis of corn waste (corn cob) was determined using AOAC methods. The bacteria were isolated and identified using serial dilution method, pour plating technique, culturing, subculturing using streaking method, gram staining technique, biochemical tests etc. Physicochemical analyses such as titratable acidity and specific gravity were determined using AOAC methods. The corn waste was hydrolyzed using H_2SO_4. The hydrolyzed sample was neutralized using 1% NaOH. Ten grams of the corn waste was added and then fermented for 96hours. From the results, the composition of corn cob for cellulose, hemicellulose, lignin, moisture, ash, protein, fat and starch were 44.02%, 32.72%, 11.30%, 6.02%, 2.23%, 2.89%, 0.30% and 0.54% respectively. The bacteria isolated and identified were *Lactobacillus casei, Gluconobacter frateuri* and *Pseudomonas aeruginosa.* The titratable acidity increased from 0.02mol/dm^3 to 0.06mol/dm^3. The specific gravity decreased from 1.0010 to 0.9641. This study shows that corn waste (corn cob) contain vital nutrients which support the growth of bacteria that are of industrial importance in the production of lactic acids. Hence it is recommended for industrial production of lactic acids.
Key words: Proximate analysis, cellulose, hemicellulose, titratable acidity, organic acids.

INTRODUCTION

Corn waste is an agricultural waste that is a potential source of energy. In Nigeria, for instance, large quantities of these wastes are generated and are vastly underutilized. The practice used is to burn the waste or leave them to decompose in the soil (Soltes, 2000). Corn waste comprises of the leaves, stalk and corn cobs. Corn cobs are mainly used as manure for agricultural production. Modern technology can be used to convert such substrates in the production of chemicals and fuels, utilizing microorganisms (Latif and Rajoka (2001); Ingram *et al.* (1998).

These wastes consist of cellulose, hemicellulose and lignin which are bonded together by covalent bond (Sullivan, 2007). Corn waste is a source of fermentable sugar because of their high availability about 80 to 100 million dry tones per year (Foyle *et al*., 2007). The basic building block of cellulose is cellobiose which is a glucose-glucose dimer. Cellulose is about 40-45% of total feedstock dry matter (Perez, *et al*., 2002). It is a glucose polymer linked by β-1,4 glycosidic bonds. Cellulose has a strong tendency to form inter and intra-molecular hydrogen bonds by the hydroxyl groups on the linear cellulose chains. This stiffens the straight chain to promote aggregation into a crystalline structure and gives cellulose a multitude of partially crystalline fiber structure and morphologies (Mod *et al*., 1981). Hemicellulose is biodegraded to monomeric sugars and acetic acid (Perez *et al*., 2002).

Various microorganisms possess the ability to convert carbohydrate to high yields of organic acids. The ability of microorganisms to convert inexpensive raw materials or substrates to economically valuable organic compounds is of considerable concern to the industrial microbiologist (Casida, 2009). Bacteria are found in decomposing plants like corn waste producing acid as the major metabolic product of carbohydrate fermentation. Many bacteria are used for commercial and industrial production of chemicals, enzymes and other bioactive molecules. For instance, acetic acid is produced by bacterium *Acetobacter aceti* and other acetic acid bacteria, lactic acid is produced by *Lactobacillus* and other lactic acid bacteria, and butyric acid is produced by bacterium *Clostridium butyricum* (Ogbulie and Nwakanma, 2015) and 2-ketogluconic acid is produced by *Pseudomonas* species (Casida, 2009).

These bacteria have special characteristic to show high tolerance to low pH range (Gottshalk, 1986).

Organic acids are produced through metabolism of carbohydrates. This organic acid accumulates in broth of fermenter, wherefrom they are separated and purified. Organic acids are either the terminal products of glycolysis (for example, lactic acid and propionic acid) or the products of incomplete oxidation of sugars (for example citric acid, itaconic acid and gluconic acid). The third type of product is obtained from the dehydrogenation of alcohol in the presence of oxygen for example acetic acid (Riviere, 1977). This work is aimed to evaluate the bacteria present in corn waste (corn cob).

MATERIALS AND METHODS

Sample collection and processing

Corn cobs were collected from a farmer in Odukpani local government area in Cross River State, Nigeria using a sterile bag and transported to Cross River University of Technology (CRUTECH) Calabar Microbiology laboratory for analysis. The corn cob was chopped and dried. The dried corn cob was ground to a powder form using IMA corn grinding mill 1A (RS 15000). The ground corn cob was sieved to produce a uniform particle size and kept in a sealed plastic jar at temperature of 30^0C (Ohimor *et al*., 2016). The sample was labeled as corn waste (corn cob).

Proximate analysis:

The proximate composition of corn cob was done in chemistry laboratory Cross River University of Technology (CRUTECH) Calabar, Nigeria. The sieved corn cob weighed 500g and used for analysis. The compositional analysis include moisture content, protein content, fat content, ash content, starch content, cellulose, hemicellulose and lignin content in the corn cob. Association of Official and Analytical Chemists (1990) methods were used.

Method for moisture content and ash content

Two grams (2g) of the sieved corn cob was weighed into a crucible of known weight. The corn cob was dried until constant weight at 105^0C for 24hours in an oven. After 24hours, the sample was removed and kept in a desiccator for cooling. It was weighed to obtain the moisture content. This was done with three different crucibles and the mean calculated. For ash content examination, three crucibles of known weight each containing 4g of the sieved corn cob was heated at 550^0C for 2hours in a furnance. After 2hours, the crucibles were removed and kept in a desiccator for cooling. It was weighed to obtain the ash content. This was done with three different crucibles and the mean calculated.

Starch content

The starch content was determined by the use of Megazyme (K-TSTA-100A) test kit according to AOAC (1990) method. This test kit is used for the measurement and analysis of total starch in cereal flours and food products. It contains improved α-amylase that allows the amylase incubation to be performed at pH 5.0 (as well as pH 7.0). The assay is done with Spectrophotometer using absorbance at 510nm.

Protein content

The analysis of protein was done with nitrogen determined indirectly by combustion method. Five gram (5g) of the sieved corn cob was used for the analysis according to AOAC (1990).

Fat content

A quantitative analysis was used to determine the fat content (chloroform methanol extraction method, gravimetric detection). It was done using 10g of the sieved corn cob by Soxhlet extraction with ether as extracting solvent. Corn cob was weighed into a thimble and 150ml of ether was measured into 250ml flat bottom flask of known weight. Soxhlet extractor which carried the thimble was fitted into the flask then, a condenser was connected to tap water inlet as the ether in flask was heated at 40^0C using hot plate.

It evaporated to the condenser and dropped back into the flask until the thimble and its content was colorless. The ether content was evaporated using water bath to obtain the fat content in the corn cob. Measurement was made to obtain weight of the fat from the corn cob as follows through gravimetric analysis after extraction (AOAC, 1990).

% fat = (weight of fat/weight of sample) x 100/1

Fiber content

The fiber content includes cellulose, hemicellulose and lignin. These contents were determined gravimetrically. Three grams (3g) of the corn cob was loaded into cellulose thimble. With the Soxhlet extractor set up, 150ml of acetone was used as solvent for extraction. It was allowed to boil at 70^0C for 4hours. After extraction, the sample was dried in an oven at 105^0C. The percentage (w/w) of the extractives content was evaluated as the difference in weight between the raw extractive-laden biomass and extractive-free biomass (Lin *et al*., 2010).

Hemicellulose was determined using 1g of extracted dried corn cob. This 1g was transferred into a 250ml Erlenmeyer flask and 150ml of NaOH was added. The mixture was boiled in a water bath for 4hours. It was allowed to cool then, filtered through a vacuum filtration and washed until neutral pH. The residue was dried at 105^0C in an oven. The difference between the sample weight before and after this treatment is the hemicellulose content (%w/w) of dry corn cob (Blasi *et al*., 1999).

For lignin content, 0.3g of dried extracted raw corn cob was weighed in glass test tube and 3ml of 72% H_2SO_4 was added. The sample was kept at room temperature for 2hours with gentle shaking at 30minutes intervals to allow for complete hydrolysis. After hydrolysis, 84ml of distilled water was added. The second step of hydrolysis was made to take place in an autoclave for 1hour at 121^0C. The slurry was allowed to cool to 30^0C. Hydrolysates were filtered through vacuum using a filtering crucible. The acid insoluble lignin was determined by drying the residues at 105^0C. The acid soluble lignin was determined by measuring the absorbance of the acid hydrolyzed sample at 320nm. The lignin content was calculated as the summation of acid soluble lignin and acid insoluble lignin (Sluiter *et al*., 2008).

Isolation, characterization and identification of bacteria in corn waste

Standard microbiological procedure (serial dilution method, pour plating technique, culturing, subculturing using streaking method, gram staining etc) for isolation, characterization, purification and identification of bacteria was used. For this study, 1.0g of the corn waste was mixed in 10ml of sterile water to obtain a solution then, 1ml of the solution (corn waste and water) was used for serial dilution and dilutions 10^{-4} and 10^{-5} were plated using pour plate technique. The bacterial isolates were purified by subculturing onto pure agar plates by streaking method. The purified isolates were characterized based on macroscopic features, gram reaction, shape of cell, motility test, and biochemical tests (catalase, oxidase, urease, coagulase, nitrate, methyl red, voges proskauer, indole, citrate, glucose, sucrose, lactose and maltose) as described by Oyeleke and Manga (2008); Cheesbrough (2003). The isolates were identified using dichotomous key as biochemical and morphological profile were obtained for each isolates. The isolates were further identified with reference to the Bergey's Manual of Determinative Bacteriology (Holt *et al*., 1994). Molecular identification method (DNA extraction, polymerase chain reaction and gel electrophoresis) was also used to properly identify the isolates after biochemical tests. This was done in International Institute of Tropical Agriculture (IITA) Ibadan for rRNA gene sequencing. Bacterial DNA was extracted and isolated using gene extraction method. The ribosomal RNA gene was amplified using polymerase chain reaction (PCR) technique. PCR products were purified and sequenced using a PCR purification kit.

The purified PCR products were reconfirmed by gel electrophoresis with 1% agarose gel. Sequences were analyzed using BLAST from National Centre of Biotechnology Information (NCBI) website (Sambrook *et al*., 2001).

Physicochemical analysis

One hundred gram of the corn cob was mixed in 1000ml sterile water then, hydrolysis was done using 5% concentrated H_2SO_4 acid. After hydrolysis, the hydrolyzed sample was neutralized using 1% NaOH. Ten grams of the corn waste was inoculated into the processed sample as source of indigenous bacteria. It was covered properly and allowed to ferment for 96hours.

The method by the AOAC (1990) was used for physicochemical analysis. The processed material was analyzed for titratable acidity and specific gravity. Titratable acidity was done using acid-base titration. The titration was done with 25ml of the sample (acid), sodium hydroxide (NaOH) was the base used and methyl red was the indicator. The titration was done in triplicates and the average titre value recorded.

Specific gravity determines the amount of fermentable sugars in the sample. A clean and dry density bottle of capacity of 50ml was weighed with its stopper. It was filled with distilled water and stoppered. The excess water was wiped with a cloth, and weighed. The bottle was emptied and dried. It was filled with the fermented broth and reweighed. This was done in triplicates and the mean recorded. Titratable acidity and specific gravity were done at 24hours intervals within the period of 96hours.

RESULTS

The bacterial isolates include *Lactobacillus casei, Gluconobacter frateuri* and *Pseudomonas aeruginosa*. The proximate composition of corn cob was found to have high contents of cellulose 44.02%, hemicellulose 32.72% and 11.30% for lignin. Other constituents are in small quantities with fat having the lowest content of 0.3%. Titratable acidity was observed to increase with increase in fermentation time while the specific gravity decreased as fermentation time increased.

Table 1: Characterization and Identification of Bacterial Isolates

S/No	Morphology and Biochemical test	Isolate A	Isolate B	Isolate C
1	Macroscopic features	Round creamy white colony	Large white colony with regular edge	Bluish green colony
2	Gram reaction	+	-	-
3	Shape of cell	Rods	Rods	Rods
4	Motility	-	+	+
5	Catalase	-	+	+
6	Oxidase	+/-	+	+
7	Urease	-	-	-
8	Coagulase	-	-	-
9	Nitrate	-	-	+
10	Methyl red	+	-	-
11	Voges proskauer	+	-	-
12	Indole	-	-	-
13	Citrate	-	-	+
14	Glucose	+	+	-
15	Sucrose	+	+	-
16	Lactose	-	-	-
17	Maltose	+	+	-
18	Identified bacteria	*Lactobacillus casei*	*Gluconobacter frateuri*	*Pseudomonas aeruginosa*

Keynotes: (+) = positive, (-) = negative

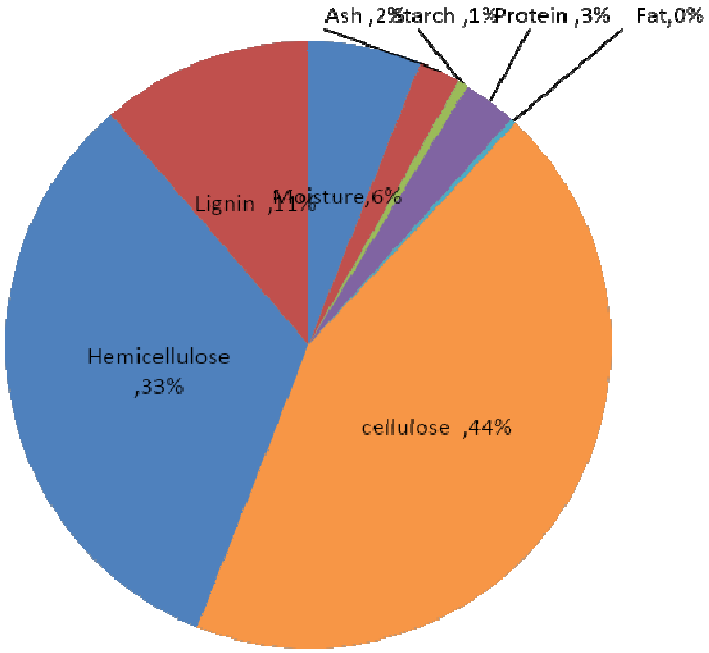

Figure 1: Proximate composition of corn cob

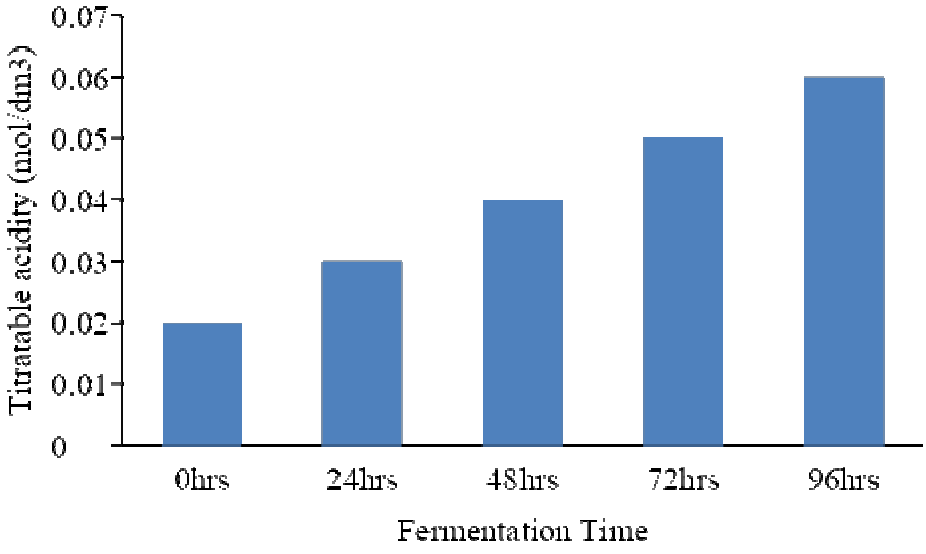

Figure 2: Titratable acidity of the fermented broth

Table 2: specific gravity of the fermented broth

S/No	Fermentation time (hours)	Specific gravity
1	24	1.0010
2	48	0.9982
3	72	0.9863
4	96	0.9641

DISSCUSSION

The characterization of the indigenous bacterial isolates from the sample showed the morphological and biochemical characteristics of the isolates. The finding from this study was compared with Bergey's manual of determinative bacteriology and the bacterial isolates were *Lactobacillus casei, Gluconobacter frateuri and Pseudomonas aeruginosa.*

The genera of bacteria isolated are those that are capable of producing acids. Jay *et al.* (2005) reported that during fermentation, lactic acid bacteria produce lactic acid. This is similar to the result in this study. It is this lactic acid that leads to the observed decrease in pH due to increased counts of lactic acid bacteria and their subsequent production of lactic acid as the end product of metabolism of sugar in the medium.

Lactobacillus casei is a lactic acid bacterium that produces lactic acid as the major end product of fermentation of carbohydrates. They convert carbohydrate to lactic acid plus carbon dioxide and other organic acids without the need for oxygen. Hatti *et al.* (2018) reported that lactic acid bacteria are used by industries as starter cultures for production of chemicals and this correlates with the result gotten in this study where lactic acid bacteria was isolated from corn waste during the production of acid.

Gluconobacter frateuri is an acetic acid bacteria, this organism was found in the corn cob because of the presence of glucose. They oxidize sugar or ethanol and produce acetic acid during fermentation. The increase in acid content is due to the presence of *Gluconobacter frateuri.* Casida (2009) revealed that species of *Pseudomonas* oxidize glucose to gluconic acids. *Pseudomonas aeruginosa* isolated in this study can be one of the species that oxidize glucose to gluconic acid as revealed by Casida (2009).

The proximate composition of corn cob has cellulose as the highest content followed by hemicellulose then lignin and others as observed in this study which correlates with the work of Kuhad and Singh (1993). The composition of corn cob shows that corn cob is a potential biomass for production of chemicals. It contains water (moisture content of about 6.02%) that helps in decomposition and metabolic activities of microorganisms.

The level of titratable acidity during fermentation of corn waste was observed to increase progressively. This result corresponds to the report of Obueh and Ikenebomeh (2014). This increase in acidity could be as a result of production of organic acids as metabolic products. Jay *et al.* (2005) also reported that during fermentation, lactic acid bacteria produce lactic acid. This further confirms the result gotten for titratable acidity.

The specific gravity in this study decreased with increase in fermentation time. The report of Braide *et al.* (2016), also confirmed that the reducing sugar decreased appreciably as the specific gravity decreases to the end of fermentation. This agrees with the results obtained in this study where the specific gravity and reducing sugar decreased with increase in fermentation time. The reason for the decrease is as a result of decrease in total soluble solids (also observed in this study) as sugar present in the broth was converted to organic acids. The results in figure 2, shows the pattern of acid production during fermentation period. The titratable acidity in the fermentation broth was observed to increase continuously from $0.02mol/dm^3$ $0.03mol/dm^3$, $0.04mol/dm^3$, $0.05mol/dm^3$, $0.06mol/dm^3$ with increase in fermentation time. The continuous increase in the production of acid in this research shows that bacteria present in corn waste and also the use of corn waste as substrate are economically viable for acid production. Despite economic difficulties, corn wastes are renewable lignocellulosic raw materials which have low cost and do not compete with food and feed chain thereby stimulating the sustainability, hence, it is suitable for industrial production of acids.

CONCLUSION

Corn waste inhabits bacteria that are degraders of cellulose and hemicellulose. Bacteria degrade the components of corn waste to produce acids as by-products of metabolism. The increase in titratable acidity shows that corn waste can be useful in the production of acids in large scale. Corn waste are abundant and available after corn harvest and consumption therefore, industries should be encourage to use corn waste rather than using corn food for acid production since the cost is low. This will offer solution to waste management and also reduce competition for food.

REFERNCES

AOAC (1990). Official Methods of Analysis of AOAC International, 16th ed. Association of Official Analytical Chemists. Washington, D.C. USA.

Blasi, C.D., Signorelli, G., Russo, C.D., Rea, G. (1999). Product distribution from pyrolysis of wood and agricultural residues. *Industrial Engineering Chemistry Research*, 38(6): 2216-2224.

Braide, W., Kanu, I., Oranusi, U. and Adeleye, S. (2016). Production of Bioethanol from Agricultural Waste. *Journal of Fundamental and Applied Sciences,* 8(2): 372-384.

Casida, L.E. (2009). Organic acids in: *Industrial Microbiology*. New age international Ltd. India. P. 402-412.

Cheesbrough, M. (2003). Medical Laboratory Manual. Tropical Health Technology. Low priced Edition. Doddington, Cambridgeshire, England. Pp 20-35.

Foyle, T., Jennings, L. and Mulchahy, P. (2007). Compositional analysis of lignocellulosic materials: Evaluation of methods used for sugar analysis of waste paper and straw, *Bioresource Technology*, 98 : 3026-3036.

Gottshalk, G. (1986). Bacterial metabolism. Springer-Verlag, New York, 2nd ed. P. 214-224.

Hatti, R., Chen, L., Dishisha, T. and Enshasy E. (2018). Lactic acid bacteria: from starter cultures to producers of chemicals. *FEMS Microbiology letters* 365 (20).

Holt, J., Krieg, N., Sneath, P., Stanley J. and Williams, S. (1994) Bergey's manual of systematic bacteriology. 9th ed. Williams and Wilkins Co. Baltimore, Maryland P.786.

Ingram, L., Gomez, P. and Lai, X. (1998). Metabolic engineering of bacteria for ethanol production. *Biotechnology and Bioengineering,* 58 (2-3): 204-214.

Jay, M.J., Loessner, M.J., Golden, A.D. (2005). Modern Food Microbiology 7th ed. *Springer science and Business media Inc.* New York, USA, p. 9-12.

Kuhad, R.C. and Singh, A. (1993). Lignocellulose Biotechnology : current and future prospects. *Critical Reviews in Biotechnology* 13:151-172.

Latif, F. and Rajoka B. (2001). Production of ethanol and Xylitol from corn cobs by yeasts. *Bioresources Technology,* 77: 57-63.

Lin, L., Yan, R., Liu, Y. and Jiang, W. (2010). In-depth investigation of enzymatic hydrolysis of biomass wastes based on three major components: cellulose, hemicellulose, and lignin, *Bioresource Technology*, 101 (21): 8217-8223.

Mod, R., Ory, R., Morris, N. and Normand, F. (1981). Chemical properties and interactions of rice hemicellulose with trace minerals in vitro, *Journal of Agriculture and Food Chemistry*, 29: 449-454.

Obueh, H.O. and Ikenebomeh, M.J. (2014). Bioethanol production and compositional changes during fermentation of cassava processing wastes from a local cassava mill. *International Journal of Current*

Research in Biosciences and Plant biology, 1(4): 43-51.

Ogbulie, T.E. and Nwakanma, C. (2015). Microorganisms and man In: Essential Analytical methods in biotechnology. WEBSmedia communication, Nigeria, p. 1-28.

Ohimor, E.O., Ndirika, V.I., Eke, A.B. and Nsidibe-Obong, E.M. (2016). Bioethanol production from corn stover using *Saccharomyces cerevisiae*. *International Journal of Scientific & Engineering Research* 7 (8): 290-293.

Oyeleke, S.B. and Manga, B.S. (2008). Essential of laboratory practical in microbiology (1st edition), Tobest, Minna Nigeria pp. 36-39.

Perez, J., Munoz-Dorado, J., Rubia, T. and Martinez, J. (2002). Biodegradation and biological treatments of cellulose, hemicellulose and lignin: an overview. *International journal of Microbiology*, 5: 53-63.

Riviere, J. (1977). *Industrial Application of Microbiology*, (Translated and edited by Moss M.O. and Smith, J.E.), Surrey University press, London, p.32.

Sambrook, J., Maccallum, P. and Russell, D. (2001). Molecular Cloning: A Laboratory Manual 3rd. Cold Spring Harbor Press, New York, p.2-141.

Sluiter, A., Hames, B., Ruiz, R., Scarlata, J. and Templeton, D. (2008). Determination of structural carbohydrates and lignin in biomass: laboratory analytical procedure. National Renewable Energy Laboratory Report, p. 510-542.

Soltes, E. (2000). Thermo Chemical routes to chemical fuels and energy from forestry and agricultural residues.4th ed. Plenum Press, New York, p. 23-47.

Sullivan, A.C. (2007). Cellulose: the structure slowly unravels, *Cellulose* 4(3): 73-207.