Qatar Univ. Sci. J. (1993), 13(2): 226 - 231

MICROBIAL UTILIZATION OF SOME AGRICULTURAL AND AGRO-INDUSTRIAL WASTE PRODUCTS FOR THE PRODUCTION OF SINGLE CELL PROTEIN (SCP).

By

N. A. A. ABO-HAMED
Department of Biology, Faculty of Education, Branch of Fayoum, University of Cairo

استخدام بعض المخلفات الزراعية والزراعية الصناعية بواسطة الميكروبات لانتاج بروتين وحيد الخلية

نبيل أبو القاسم أبو حامد

تم تحضير منبت غذائي يحتوي على نسب مختلفة من مخلف قش الذرة الذي أجريت له عملية تحلل مائي بواسطة حمض الكبريتيك (٠.٥ ع) ، وتركت فطرة الخميرة « سكاروميسيس سيريفيزي» لتنمو عليه بعد تهيئة كافة الظروف المناسبة للنمو من درجة حرارة ودرجة تركيز أيون الهيدروجين . وبعد انتهاء فترة التحضين أخذت عينات من المنبت ومن خلايا الخميرة وحللت . وأظهرت نتائج التحليل أن أكبر كمية من الوزن الحيوي المحتوي على أعلى نسبة من البروتين الخلوى تم الحصول عليها من المنبت المحتوي على كمية من المخلف بها نسبة من السكر مقدارها ١٥٪ . وفي الجزء الثاني من هذا البحث أخذ هذا المنبت وخلط مع تركيزات مختلفة من المولاس ، وتركت الخميرة لتنمو على تلك المنابت . وقد أظهرت نتائج التحاليل أن أكبر ناتج من الكتلة الحيوية للخميرة والذي يحتوي على أعلى نسبة من البروتين الخلوي قد تكون في المنبت الذي يحتوي على نسبة من المولاس قدرها ٢ جرام / لتر . و من ثم ، فإنه يمكن انماء الخميرة على منبت غذائي يتكون من مخلف قش الذرة (بنسبة ١٥٪ سكر) والمولاس (بنسبة ٢ جرام / لتر) لتعطى أعلى ناتج من الوزن الحيوى المحتوي على أكبر نسبة من البروتين الخلوي .

Key Words: Agricultural waste, Agro-industrial waste, Single cell protein, Microbial utilization.

ABSTRACT

The dried residues of corn crop (corn straw) were hydrolysed by 0.5 NH_2SO_4. *Saccharomyces cerevisiae* has been allowed to grow on media containing different concentrations of the corn straw hydrolysate (CSH). The optimal concentration of CSH at which the maximal biomass gain with the higher content of cellular protein (SCP) was that containing 15% sugars. To this medium cane molasses (CM) were added in different concentrations, and *S. cerevisiae* has been grown on it. the maximal biomass containing the highest amount of SCP was yielded in case of the medium containing CM in 2 g/L concentration.

INTRODUCTION

As the need for single cell protein (SCP) increases more attention has been constructed for the usage of unconventional substances as new substrates for the fermentation processes. Of these substance, agricultural wastes have been used by many investigators resulting in converting these wastes into commercially valiable products, especially single cell protein (Peitersen, 1975; Riviere *et al.*, 1978; Moriguchi, 1982; Maurizio and Federico, 1986 and Jwanny *et al.*, 1990). Some investigators used beet and cane molasses as a substrate for the fermentation process on which the micro-organisms have been grown (Peppler, 1968; Gazaerly, 1983; Olama, 1985; El-Refai *et al.*, 1986 and Abo-Hamed, 1987). In this investigation we used corn straw hydrolysate in addition to cane molasses, in an attempt to obtain a substrate consisting of a mixture of these two wastes on which the yeast *Saccharomyces cerevisiae* grows for the production of a cell biomass containing the highest amount of single cell protein. Cane molasses is produced in Egypt in large quantities as a cheap by-product in sugar industries. Corn straw is also an agricultural waste product produced in appreciable amounts in Egypt as well as in many countries of the world. The usage of these cheap agricultural wastes for

the production of microbial feed and fodder protein was the aim of this work.

MATERIALS AND METHODS

Maintenance and preparation of the inoculum

Saccharomyces cerevisiae was isolated from local commercial baker's yeast. The cultures were maintained on slopes of Phaff's medium of the following composition (g/l): D-glucose, 10; $(NH_4)_2 SO_4$, 5.0; KH_2PO_4, 1.0; $MgSO_4 \cdot 7H_2O$, 0.5; NaCl, 0.1; $Ca Cl_2$, 0.1; pH 5.5. Before inoculation, the organisms were serially subcultured for at least three times on the same medium which was used also as a control and basal medium.

Fermentation media

a) Corn straw hydrolysate medium (CSH):

Corn straw was dried at 80° C and hyrolysed with 0.5 N H_2SO_4 at 121° C for 20 minutes. The reducing sugar's content of this hydrolysate was 34.7%. Seven sets of media have been prepared, each set consists of 3 replicates, the sugar content of which was:

1. 5% sugar equals 14.4% straw hydrolysate.
2. 10% " " 28.8% " ".
3. 15% " " 43.2% " ".
4. 20% " " 57.6% " ".
5. 25% " " 72.0% " ".
6. 30% " " 86.4 " ".
7. Control (basal medium): " ".

b) Cane molasses medium (CM)

Cane molasses has been obtained from "The Egyptian sugar and Distillary Company" (Hawamdia Factory, Giza Egypt). Molasses were analysed to determine their chemical composition before use (Table 1). Six sets of flasks (each consists of 3 flasks) have been prepared, and cane molasses (CM) were mixed with the optimum concentration of corn straw hydrolysate (CSH) in the following manner:

1. Control : (CSH) 43.2% in distilled water.
2. CM : 1 g/l CSH.
3. CM : 2 " ".
4. CM : 3 " ".
5. CM : 4 " ".
6. CM : 5 " ".

Table 1
Chemical composition of sugar cane molasses (mg/100 mg fresh molasses).

Constitutens	mg/100 mg cane molasses
Water content	21.57
Total solids	78.43
Ash	11.84
Total sugars	54.62
Ammonia - N	0.207
Amino acids - N	0.488

Table 1 Contd.

Constitutens	mg/100 mg cane molasses
Peptides - N	0.672
Total soluble - N	1.367
Insoluble N	0.154
Total N	1.521
Protein	9.505

pH was adjusted at 5.5 and the media were distributed in 50 ml amounts in Erlenmeyer flasks and sterilized at 121° C for 15 minutes before inoculation.

Cultivation:

The yeast cells of age 48 hours were suspended in sterile distilled water to an optical density of 2.5 at 400 n.m. (measured on Spekol spectrophotometer). The flasks have been inoculated each with 5% (V/V) cell suspension and incubated at 28° C on a rotary shaker at 180 r.p.m. for 6 days. At the end of the fermentation period, the cells were separated by centrifugation at 10,000 r.p.m. for 20 minutes, washed and kept in the refrigerator until analysis.

Analyses:

Growth of the yeast cells was estimated by drying the separated cells (after washing) at 70° C to a constant weight. The original, as well as the unassimilated sugars were determined in the media as glucose, according to the method described by Dubois *et al.*, (1956). The yeast cells were analysed for their protein content using the procedure mentioned by Fawcett and Scott (1960). The cells were further analysed for their content of ammonia (Chaney and Marbach, 1962), amino acids (Russel, 1944), peptides (Lowry *et al.*, 1951), total soluble nitrogen and total nitrogen (Fawcett and Scott, 1960). The obtained data were statistically analysed by using F test, Duncan's method and L.S.D. test (Steel and Torrie, 1980).

RESULTS

a) Selection of the suitable corn straw hydrolysate (\CSH) concentration:

In the first part of this investigation wherever the corn straw hydrolysate (CSH) only was used as a sole carbon and nitrogen source, we observed that the sugar utilization increased in presence of the low and moderate concentrations of the straw hydrolysate. Maximal amount of utilized sugar (491 mg/100 ml) occurred in presence of the waste concentration which contains 15% reducing sugars (medium No. IV), (Table 2). In presence of the higher waste concentrations, sugar utilization decreased. The minimal amount of utilized sugar (276.5 mg/100 ml) occurred in presence of the highest waste concentration (medium No. VII). The same observation has been found with respect to nitrogen consumption which increased with the increase of the waste concentration in the fermentation media till it reached its maximal value (47.9 mg/100 ml) in the medium containing 43.2% waste which equals 15% sugar (medium No. III). Increasing the waste concentration in the fermentation media over this concentration led to a decrease in the amount of utilized N.

Table 3 shows the characteristics of the yeast biomass at the end of the fermentation process. It is evident that the presence of the lowest and highest waste concentrations in the fermentation media lowered the amount of cellular N fractions under control, while the moderate concentrations of

Table 2
Changes in corn straw hydrolysate (CSH) media inoculated with *Saccharomyces cerevisiae* throughout the fermentation period. (amounts are represented as mg/100 ml).

Fermentation media	Total reducing sugars				Total nitrogen				pH	
	Initial (mg)	Final (mg)	Uptake (mg)	Uptake (%)	Initial (mg)	Final (mg)	Uptake (mg)	Uptake (%)	Initial	Final
I	998.4	611.2	387.2	38.8	46.94	9.81	37.13	79.1	5.5	4.0
II	489.2	136.4	352.8	72.1	24.74	2.66	22.08	89.25	5.5	3.9
III	997.7	520.1	477.6	47.9	49.51	9.97	39.57	79.92	5.5	3.9
IV	1494.4	1003.4	491.0	32.8	73.28	25.38	47.9	65.4	5.5	3.8
V	1996.1	1649.1	347.0	17.4	97.62	60.92	36.7	37.6	5.5	3.8
VI	2484.0	2155.7	328.3	13.2	122.41	89.01	33.4	27.3	5.5	3.9
VII	2996.0	2719.5	276.5	9.2	146.48	177.88	28.6	19.5	5.5	4.0

I Control (basal medium).
II CSH 14.4%, 5% sugar.
III CSH 28.8%, 10% ".
IV CSH 43.2%, 15% ".
V CSH 57.6%, 20% ".
VI CSH 72.0%, 25% ".
VII CSH 86.4%, 30% ".
CSH: Corn Straw Hydrolysate

the waste increased the amounts of these cellular N fractions. Maximal increase of ammonia (35.5%), amino acids (58.9%), peptides (39.6%), and total N (40.6%) have been attained in the medium No. IV which contains the moderate concentration of waste (43.2% & 15% sugars). At the same waste concentration, the maximal gain of biomass (64.41 mg/100 ml) was yielded, raising over control by 29.5% and containing the higher cellular protein (SCP) (21.14 mg/100 ml) with a 40.5% increase over control (Table 3).

Table 3
Biomass characteristics of *Saccharomyces cerevisiae* grown on corn straw hydrolysate (CSH) media throughout the fermentation period. (amounts are represented as mg/100 ml).

Item		Fermentation media							L.S.D.	
		I	II	III	IV	V	VI	VII	0.01	0.05
Ammonia	Amount	0.062	0.041	0.076	0.084	0.069	0.033	0.027	0.038	0.026
	% Change	-	(-) 33.9	(+) 22.6	(+) 35.5	(+) 11.3	(-) 46.8	(-) 56.5		
Amino acids	Amount	0.124	0.092	0.136	0.197	0.103	0.075	0.051	0.081	0.055
	% Change	-	(-) 25.8	(+) 9.7	(+) 58.9	(-) 16.9	(-) 39.5	(-) 58.9		
Peptides	Amount	1.978	1.224	1.989	2.761	1.474	0.966	0.575	1.25	0.85
	% Change	-	(-) 38.1	(+) 0.56	(+) 39.6	(-) 25.5	(-) 51.2	(-) 70.9		
Total soluble N	Amount	2.164	1.357	2.201	3.042	1.646	1.074	0.653	1.36	0.93
	% Change	-	(-) 37.3	(+) 1.7	(+) 40.6	(-) 23.9	(-) 50.4	(-) 70.7		
Insoluble N	Amount	0.243	0.152	0.246	0.340	0.183	0.117	0.076	0.147	0.100
	% Change	-	(-) 37.4	(+) 1.2	(+) 39.9	(-) 24.7	(-) 51.9	(-) 68.7		
Total N	Amount	2.407	1.509	2.447	3.382	1.829	1.191	0.729	1.53	1.04
	% Change	-	(-) 37.3	(+)1.7	(+) 40.5	(-) 24	(-) 50.5	(-) 69.7		
Dry weight of biomass	Amount	49.72	31.54	50.2	64.41	38.47	25.92	16.15	2.81	1.91
	% Change	-	(-) 36.6	(+) 0.96	(+) 29.5	(-) 22.6	(-) 47.9	(-) 67.5		
SCP	Amount	15.04	9.43	15.29	21.14	11.43	7.44	4.56	0.95	0.65
	% Change	-	(-) 37.3	(+) 1.7	(+) 40.5	(-) 23.9	(-) 50.5	(-) 69.7		

Table 3 Contd.

Item		Fermentation media							L.S.D.	
		I	II	III	IV	V	VI	VII	0.01	0.05
Protein % dry biomass	Amount	30.24	29.89	30.46	32.82	29.7	28.7	28.24		
PCC	Amount	3.8	2.7	3.2	4.3	3.3	2.3	1.6		
EC	Amount	12.8	8.9	10.5	13.1	11.1	7.9	5.8		

CSH : Corn straw hydrolysate
Medium I : Control (basal medium)
" II : CSH 14.4%, 5% sugar.
" III : CSH 28.8%, 10% ".
" IV : CSH 43.2%, 15% ".
" V : CSH 57.6%, 20% ".
" VI : CSH 72.0%, 25% ".
" VII : CSH 86.4%, 30% ".

PCC : Protein conversion coefficient

$$= \frac{\text{Weight of protein}}{\text{utilized sugar}} \times 100$$

EC : Economic coefficient

$$= \frac{\text{weight of dry biomass}}{\text{utilized sugar}} \times 100$$

SCP : Single cell protein

b) Growth-relationship to cane molasses:

In the second part of this investigation, analyses of the fermentation media which consist of corn straw hydrolysate (CSH) in addition to cane molasses (CM), were carried out. It is evident that the presence of cane molasses in the fermentation media raised the sugar uptake from these media over control except in the medium containing the highest concentration of molasses where the sugar uptake decreased than control. Maximal sugar uptake (532 mg/100 ml) occurred in the medium containing 2 g/l cane molasses, (Table 4).

With respect to N utilization, it is clear that N utilization is positively affected by the presence of cane molasses in the fermentation media. Maximal N uptake (63.54 mg/100 ml) occurred in the fermented medium containing 2 g/l cane molasses, (Table 4).

Table 4
Changes in cane molasses media (CM) inoculated with *Saccharomyces cerevisiae* throughout the fermentation period. (amounts are represented as mg/100 ml).

Medium	Total reducing sugars				Total nitrogen				pH	
	Initial (mg)	Final (mg)	Uptake (mg)	Uptake (%)	Initial (mg)	Final (mg)	Uptake (mg)	Uptake (%)	Initial	Final
I	1494	998	496	33.2	73.34	13.61	59.73	81.44	5.5	3.8
II	1549	1035	514	33.2	74.63	13.24	61.39	82.26	5.5	3.8
III	1604	1072	532	33.2	75.96	12.16	63.54	83.65	5.5	3.7
IV	1659	1147	520	31.3	77.21	15.25	61.96	80.25	5.5	3.7
V	1714	1219	506	29.5	78.53	17.31	61.22	77.96	5.5	3.7
VI	1768	1294	485	27.4	79.88	19.62	60.26	75.44	5.5	3.6
L.S.D. 0.01	2.24					5.93				
L.S.D. 0.05	1.62					4.29				

Medium I : Control (Corn straw hydrolysate CSH)
" II : CSH + 1 g/l cane molasses (CM)
" III : " + 2 " " " "
" IV : " + 3 " " " "
" V : " + 4 " " " "
" VI : " + 5 " " " "

Analyses of biomass showed that the amounts of N fractions accumulated within the yeast cells increased over control in presence of cane molasses (Table 5). The increase of N fractions was maximum in case of the media containing cane molasses in a concentration of 2 g/l. Maximal percentage increase of ammonia was (61.79%), amino acids: (50.77%), peptides: (28.32%), and total N: (30.6%) over control.

Concerning the biomass yield and its protein content, it is evident that the presence of cane molasses in the fermentation media increased the biomass yield, such increase was accompanied with a concomitant increase of cellular protein (SCP) over control (Table 5). Maximal biomass yield (76.42 mg/100 ml) with a percentage increase of 17.9% over control was reached in case of the fermentation medium containing cane molasses in 2 g/l concentration. This biomass gain contained the highest amount of protein (SCP) which was (27.84 mg/100 ml) constituting a 30.6% increase over control. As the concentration of cane molasses in the fermentation media increased, the biomass yield and its protein content decreased, but they remained higher than control. All yeast cells produced in presence of cane molasses contained percentages of cellular protein (SCP) higher than that present in case of control, but the highest percentage of SCP (38.61%) was attained in presence of 2 g/l concentration of cane molasses. Biostatistical analysis revealed the presence of significant differences between the data.

Table 5

Biomass characteristics of *Saccharomyces cerevisiae* grown on media consisting of corn straw hydrolysate (CSH) and cane molasses (CM) throughout the fermentation period (amounts are represented as mg/100 ml).

Item		Fermentation media						L.S.D.	
		I	II	III	IV	V	VI	0.01	0.05
Ammonia	Amount	0.089	0.107	0.144	0.126	0.101	0.094	0.043	0.031
	% Change	-	(+) 20.2	(+) 61.8	(+) 41.6	(+) 13.5	(+) 5.6		
Amino acids	Amount	0.195	0.236	0.294	0.272	0.251	0.205	0.076	0.055
	% Change	-	(+) 21.03	(+) 50.7	(+) 39.5	(+) 28.7	(+) 5.13		
Peptides	Amount	2.782	2.990	3.570	3.212	3.065	2.920	0.55	0.39
	% Change	-	(+) 7.48	(+) 28.32	(+) 15.46	(+) 10.17	(+) 4.96		
Total soluble N	Amount	3.066	3.333	4.008	3.610	3.417	3.219	0.65	0.47
	% Change	-	(+) 8.71	(+) 30.7	(+) 17.7	(+) 11.4	(+) 4.99		
Insoluble N	Amount	0.346	0.373	0.447	0.405	0.382	0.359	0.072	0.052
	% Change	-	(+) 2.6	(+) 29.2	(+) 17.1	(+) 10.4	(+) 3.8		
Total N	Amount	3.412	3.706	4.455	4.015	3.799	3.578	0.719	0.519
	% Change	-	(+) 8.6	(+) 30.6	(+) 17.7	(+) 11.3	(+) 4.9		
Dry weight of biomass	Amount	64.83	67.71	76.42	73.54	69.92	66.79	8.84	6.39
	% Change	-	(+) 4.4	(+) 17.9	(+) 13.4	(+) 7.9	(+) 2.5		
SCP	Amount	21.32	23.16	27.84	25.09	23.74	22.36	4.86	3.52
	% Change	-	(+) 8.6	(+) 30.6	(+) 17.7	(+) 11.3	(+) 4.9		
Protein % of dry wt.		33.1	35.12	38.61	35.98	35.68	35.71		
PCC		4.3	4.5	5.2	4.8	4.7	4.6		
EC		13.0	13.2	14.4	14.1	13.8	13.7		

Medium I : Control (CSH medium without CM)
" II : CM 1 gm per L CSH
" III : CM 2 " " " ".
" IV : CM 3 " " " ".
" V : CM 4 " " " ".
" VI : CM 5 " " " ".

CSH : Corn straw hydrolysate

CM : Cane molasses

DISCUSSION

Preliminary results indicated that *Saccharomyces cerevisiae* could grow and convert corn straw hydrolysate, as well as cane molasses, to useful products, especially single cell protein. this agrees with the findings of El-Refai *et al.*, (1986), Abo-Hamed (1987), Omobuwajo *et al.*, (1987) and Abo-Hamed (1993).

The crude protein content of the dry cells grown on the media containing moderate concentrations of corn straw hydrolysate is higher than that of the cells grown on the media containing either the low or the high concentrations of this waste. The results obtained at the low concentrations of CSH could be attributed to the fact that the nutrients present at this concentration are insufficient for the vital processes of the yeast cells. On the other hand, the decrease in both yeast biomass and its content of crude protein at the high waste concentrations may be attributed to the high osmotic pressure of the fermentation media at these high concentrations of the

waste, which hinders the yeast cells to absorb and assimilate the nutrients from these media.

The crude protein of the yeast cells grown on the fermentation media which consist of a mixture of CSH and cane molasses is higher than that of the cells grown on CSH only.

The production of a yeast biomass in a large quantity with a high level of protein content after growth of the yeast cells on fermentation media consisting of a mixture of corn straw hydrolysate (CSH) and cane molasses (CM), can be explained on the basis that cane molasses contain appreciable amounts of sugars and other nitrogen fractions which are satisfactorily assimilated by the yeast cells.

Finally, it can be concluded from the results of the present work, that corn straw hydrolysate can be utilized in a mixture with cane molasses by *Saccharomyces cerevisiae* as a carbon and nitrogen sources for the production of feed and fodder yeast rich in SCP.

REFERENCES

Abo-Hamed, N. A. A., 1987. Variability of the nature and concentration of medium constituents in relation to protein and sterol production by *Saccharomyces cerevisiae.* Ph. D. Thesis, Faculty of Science, University of Cairo.

Abo-Hamed, N. A. A., 1993. Bioconversion of wheat straw by yeasts to single cell protein. Egypt. J. Microbiol., 28: 1-9.

Alian, A. M., H. G. El-Masry, S. B. El-Magoli and **M. F. Ahmed, 1988.** Production of single cell protein from selected agro-industrial solid wastes. 1. Factors affecting growth and protein biosynthesis by *Trichoderma harzianum* using rice straw. Egypt. J. Food Sc., (Special Issue) 19-30.

Chaney, A. L. and **E. P. Marbach, 1962.** Modified reagents for determination of urea and ammonia. J. Clin. Chem., 8: 130-132.

Dubois, M., K. Gills, J. K. Hamilton, P. A. Robers and **F. Smith, 1956.** Colourimetric methods for determination of sugars and related substances. Anal. Cham., 28: 350-352.

El-Refai, A. H., K. M. Ghanem and **M. A. El-Gazaerli, 1986.** Single cell protein production from beet molasses. Microbios., 46: 95-102.

Fawcett, J. K. and **J. E. Scott, 1960.** A rapid and precise method for determination of urea. J. Clin. Path., 13: 156-159.

Gazaerly, M. A., 1983. The utilization of Beet molasses for single cell protein production. M. Sc. Thesis, Botany Dep., Faculty of Science, Alexandria University.

Jwanny, E. W., K. M. Gaffar and **M. M. Rashad, 1990.** Production and evaluation of a yeast utilizing an industrial waste. Biological wastes., 34: 313-321.

Lowry, O. H., J. Rosenbrough, A. C. Fan and **R. J. Randal, 1951.** Protein measurement with folin phenol reagent. J. Biol. Chem., 193: 265-275.

Maurizio, P. and **F. Federico, 1986.** Reninlike enzyme production of *Cryptococcus albidus* var. *aerius* on orange peel waste. Ann. Fac. Agar. Univ. Studi. Perugia, 83: 391-396.

Moriguchi, M., 1982. Fermentation production of pyruvic acid from citrus peel extract by *Debaryomyces condertii.* Agric. Biol. Chem., 46: 955-961.

Olama, Z. A., 1985. Utilization of hydrocarbons of Egyptian crude oils by some microorganisms. Ph. D. Thesis, Faculty of Science, Alexandria University, Egypt.

Omobuwajo, T. O., E. Balough and **S. K. Layokum, 1987.** The potential of corn straw for bioconversion to single cell protein. Lebensm-Wiss Technol, 20 (4): 191-194.

Peitersen, N., 1975. Production of cellulose and protein from barley straw by *Trichoderma viride.* Biotechnol. Bioenergy, 17: 361-74.

Peppler, H. J., 1968. In: Single-cell protein. (eds. R.I. Mateles and S.R. Tannenbaum) MIT press, Cambridge, Massachusetts., 229-242.

Phaff, H. J., M. W. Miller and **E. M. Mark, 1966.** the life of yeasts. Harvard University press. Cambridge, Massachusetts. p. 186.

Riviere, J., H. Heslot, M. Coudert and **J. P. Delage, 1978.** Protein production by microorganisms from ground wheat. Ann. Technol. Agric., 27: 585-607.

Russel, J. A., 1944. Note on the colorimetric determination of amino nitrogen. J. Biol. Chem., 156: 467-468.

Scringeour, M., 1978. Unconventional sources of protein. J. Human Nutrition, 32: 439.

Steel, R. G. D. and **H. J. Torrie, 1980.** Principles and procedures of Statistics. 2nd ed. Mc. Graw-Hill Kagakasha, Ltd. Tokyo, Japan.