Pak J Sci Ind Res 2000 43 (1) 26-32

EFFECT OF CARBON AND NITROGEN SOURCES ON ACETONE AND BUTANOL PRODUCTION BY A LOCAL STRAIN OF *CLOSTRIDIUM ACETOBUTYLICUM*

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(Received 27 February 1999; accepted 23 July 1999)

The role of carbon and nitrogen sources on the production of acetone and butanol by a local strain of *C. acetobutylcium* was investigated. Molasses was found to be superior as compared with the other tested mono di and polysaccharides. The highest solvent yield (17.37 g l^{-1}) was obtained with a culture medium containing 140 g l^{-1} molasses. $(NH_4)_2SO_4$ proved to be the best inorganic nitrogen source whereby a total yield of 17.7 g l^{-1} solvents were obtained at 2 g l^{-1} salt level. Among the different organic nitrogen soures tested, rice bran and corn bran mixture (5 g l^{-1} each) proved to be the best, where by 18.56 g l^{-1} of total solvents were obtained.

Key words: Molasses, Polysaccharides, *Clostridium acetobutylicum*.

Introduction

The acetone butanol fermentation has a long history as a successful industrial fermentation process. Solvent production by *C. acetobutylicum* has been studied intensively since early in this century because of the economic importance of the fermentation end products (Gottschalk and Bahl 1981; Gibbs 1983). Batch fermentation by *C. acetobutylicum* is characterized by two phases. During the first phase or acidogenesis, *C. acetobutylicum* grows and produces acetate and butyrate from glucose. These acids attain their maximal concentration and are consumed in the second phase, which is known as solventogenesis. The acids are reduced and neutral solvents including butanol, ethanol and acetone are produced (Walton and Martin 1979). Strains of *C. acetobutylicum* are known to metabolize a wide range of sugars, including the mono and di saccharides, arabinose, xylose, rhamnose, fructose, galactose, glucose, mannose, lactose and maltose (Buchanan and Gibbons 1974).

Fermentations utilizing molasses as substrate were investigated by many workers (Yassine 1977; Ayaakiishizaki 1995) using nitrogen sources: $(NH_4)_2SO_4$, NH_4Cl, NH_4NO_3 and NH_4OH (Beesch 1952). Most synthetic media use 2 to 3 g l^{-1} of $(NH_4)_2SO_4$ as the nitrogen source (Gottschal and Morris 1981). Moreover, organic sources of nitrogen and a buffering agent ($CaCO_3$) were supplemented to the molasses fermentation medium (Jones and Woods 1986). This paper presents the effect of carbon and nitrogen sources on the production of acetone and butanol by a local strain of *C. acetobutylicum*.

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Materials and Methods

Microorganism and culture conditions. The bacterium used in this study was isolated from a soil sample taken from the rhizosphere of bean roots using Weizmann's method of isolation, involving successive heat shocking (Calam 1979). Inocula were prepared by adding 0.2ml of spore suspension to 10ml of potato glucose medium anaerobic tubes with butyl bungs and aluminum crimps. Potato glucose medium contains the following ingredients (g l^{-1}): wet potato mash 250; glucose 5; $(NH_4)_2SO_4$ 3; $CaCO_3$ 1.5 and cysteine hydrochloride 0.5.

The pH was adjusted to 5.5 prior to autoclaving. The spore suspension was heat shocked at 100°C for 2 min. This culture was used to inoculate (5% v/v) 50ml of potato glucose medium in a 100 ml serum bottle. The culture was incubated overnight and then used as inocula for the experiments. Anaerobic conditions were maintained by sweeping oxygen free nitrogen gas across the surface of the culture according to the method of Hungate (Hungate 1969). Inoculum was chosen as 10% by volume and in the exponential growth phase. Fermentations were performed in 150ml serum bottles using 100ml of the fermentation medium and 10% v/v of the inoculum. The bottles were incubated at 32°C for 96 h. The fermentation medium contained the following ingredients (g l^{-1}): molasses 100; $(NH_4)_2SO_4$ 3; $CaCO_3$ 1.5; rice bran 5; corn bran 5. Cultures were continuously vented to prevent the build up of gas pressure.

Effect of the nature of the carbon source on solvent production. Different concentrations of molasses (20-180 g l^{-1}) were added to the basal fermentation medium. Also different levels of glucose, surcrose and starch (10-90 g l^{-1}) were individually supplemented to the basal fermentation medium instead of molasses.

Effect of the addition of different nitrogen sources on solvent production. Different inorganic and organic nitrogen sources were tested to examine their effect on solvent production. Different levels of each inorganic nitrogen source i.e. $(NH_4)_2SO_4$ (1-6 g l^{-1}); $(NH_4)_2HPO_4$ (0.5-5.5 g l^{-1}) and KNO_3 (2-10 g l^{-1}) were individually added to the basal fermentation medium. Also different organic nitrogen sources with different levels i.e. urea (2-10 g l^{-1}), rice bran (1-9 g l^{-1}), corn bran (1-9 g l^{-1}), fodder yeast (2-10 g l^{-1}) and yeast autolyzate (2-10 g l^{-1}) were tested.

Results and Discussion

Effect of the nature of the carbon source: Molasses. The results (Table 1) showed that the maximal output of acetone and butanol (17.37 gl^{-1}) was recorded at molasses concentration of 140 g l^{-1}.On the other hand, lower or higher levels of molasses were accompanied by smaller amounts of solvent yield.

Glucose. The results shown in (Fig 1) indicated that the optimum concentration of glucose for butanol production was 3% whereas the optimum concentration for acetone production was 4%. The maximal ouput of total solvents (8.96 g l^{-1}) was recorded at glucose concentration of 4 g%.

Sucrose. The results (Fig 2) indicated that the total solvent yield increased with the increase in sucrose concentration reaching its optimum value (6.24 g l^{-1}) at sucrose level of 6%.

Starch. The results (Fig 3) showed that the optimum solvent yield (6.8 g l^{-1}) was recorded at 3g% starch. It can be concluded from the previous 4 experiments that the experimental organism productivity was greatly affected by the type and level of the sugar employed. Molasses was found to be superior as compared with the other tested mono di polysaccharides which may be attributed to the presence of other ingredients in addition to sugars which support better solvent production. Egyptian black strap molasses contained 54% total sugars, nitrogen sources, organic acids and salts in the form of Ca, Mn, Fe, Cu, Ni, Na, K and others. Fermentation utilizing molasses as substrates was investigated by many workers (Qureshi and Maddox 1992; Quratulain *et al* 1995; Guvenilir and Deveci 1996).

Effect of the nature of inorganic nitrogen source: Ammonium sulphate. The results (Fig 4) indicated that the acetone butanol fermentation was affected by the amounts of $(NH_4)_2SO_4$ added. As the concentration of $(NH_4)_2SO_4$ increased, the amount of the total solvents also increased until it reached the maximum output (17.7 g l^{-1}) at $(NH_4)SO_4$

Table 1
Effect of different concentrations of molasses on acetonebutanol production by *C. acetobutylicum*

Molasses conc (g%)	Final pH	Acetone (mg 100ml^{-1})	Butanol (mg 100ml^{-1})	Total solvents (g 100ml^{-1})	Residual sugars (g 100ml^{-1})
2	4.45	48	224.5	272.5	0.09
4	4.57	183	329	512	0.1
6	4.70	227	628	855	0.18
8	4.90	290	744	1034	0.32
10	5.00	454	1173	1627	0.8
12	5.15	450	1229	1689	1.74
14	5.07	464	1273	1737	1.8
16	5.08	464	1232	1696	4.22
18	5.06	448	1216	1664	4.48

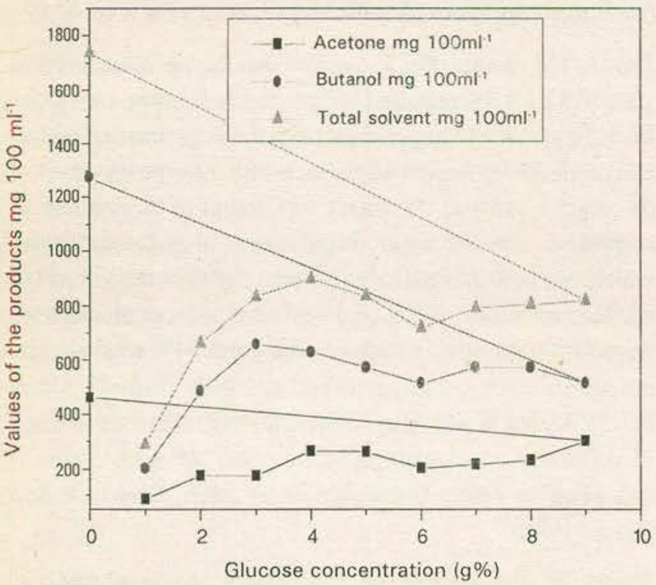
1. The medium composition (g l^{-1} wv^{-1}): molasses, different levels; $(NH_4)_2SO_4$, 3; $CaCO_3$, 1.5; rice bran, 5; corn bran, 5 at pH 5.5
2. Incubation period, 4 days at 32° C.

level of 2 g l^{-1}.

Ammonium monohydrogen phosphate. The results (Fig 5) showed that the amount of solvents increased with the increase of $(NH_4)_2HPO_4$ level reaching its optimum value at 4.5 g l^{-1}, above which a slight decrease was observed.

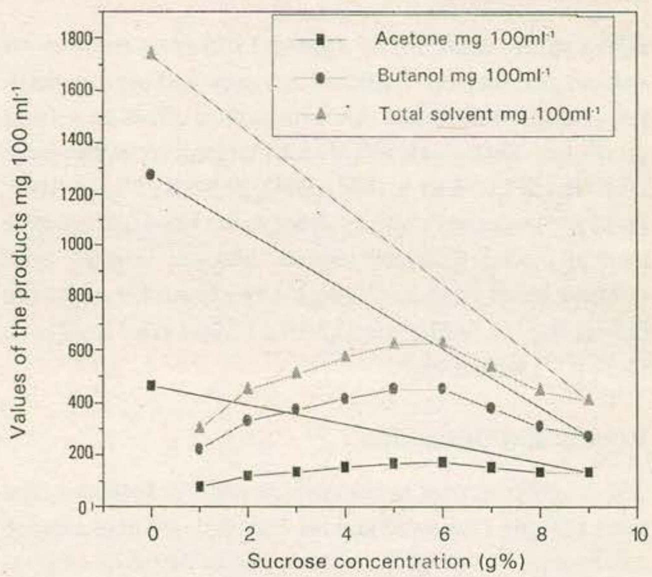
Potassium nitrate. It was observed (Fig 6) that the total neutral solvents decreased with the increase of KNO_3 concentration which indicated that, KNO_3 may have a harmful effect on *C. acetobutylicum.*

The results showed that ammonium sulphate was the best for



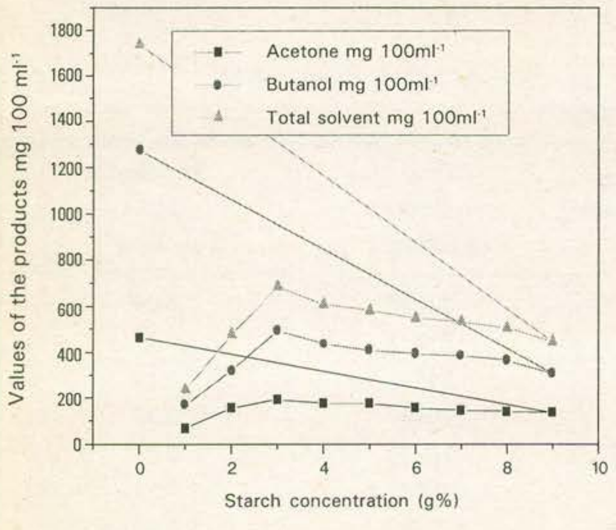
Incubation period, 4 days at 32°C; 0, control using molasses source; medium (g l^{-1}; wv^{-1}): glucose, different levels; amm. sulphate, 3; $CaCO_3$, 1.5; rice bran, 5; corn bran, 5; at pH 5.5.

Fig 1. Effect of different glucose concentrations on the production of the acetone butanol by *C. acetobutylicum.*



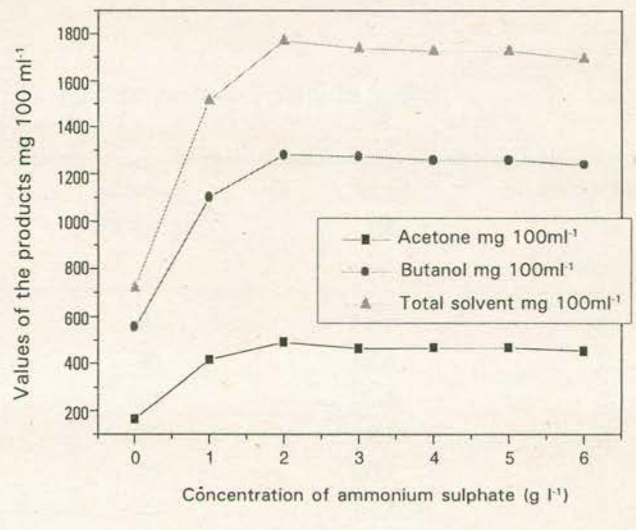
Incubation period, 4 days at 32°C; 0, control using molasses source; medium (g l^{-1}; wv^{-1}): sucrose, different levels; amm. sulphate, 3; $CaCO_3$, 1.5; rice bran, 5; corn bran, 5; at pH 5.5.

Fig 2. Effect of different sucrose concentrations on the production of the acetone butanol by *C. acetobutylicum.*



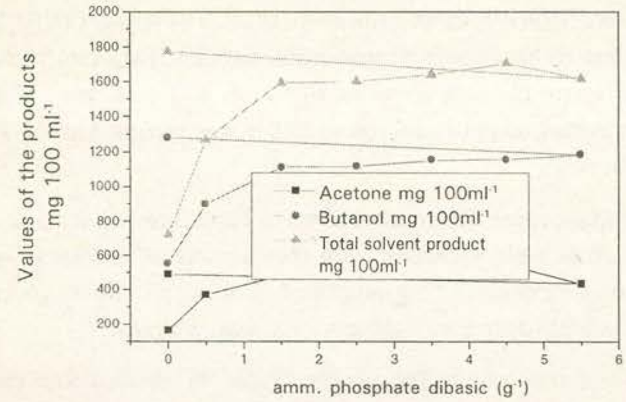
Incubation period, 4 days at 32°C; 0, control using molasses source; medium (g l^{-1}; wv^{-1}): starch, different levels; amm. sulphate, 3; $CaCO_3$, 1.5; rice bran, 5; corn bran, 5; at pH 5.5.

Fig 3. Effect of different starch concentrations on the production of the acetone butanol by *C. acetobutylicum.*



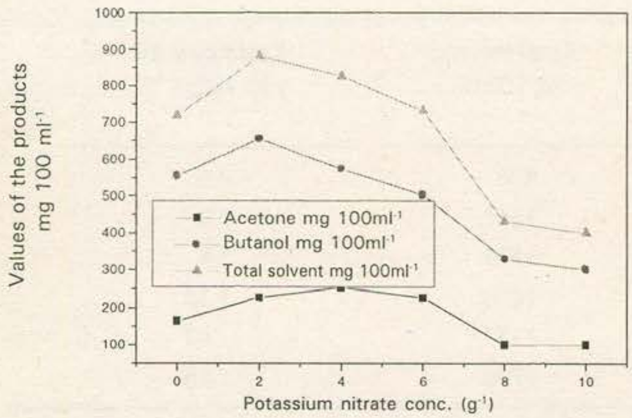
0, control amm. sulphate, 2g l^{-1}; incubation period, 4 days at 32°C; medium (g l^{-1}; wv^{-1}): molasses, 140; amm sulphate, different levels; $CaCO_3$, 1.5; rice bran, 5; corn bran, 5; at pH 5.5.

Fig 4. Effect of different concentrations of ammonium sulphate on acetone butanol production by *C. acetobutylicum.*



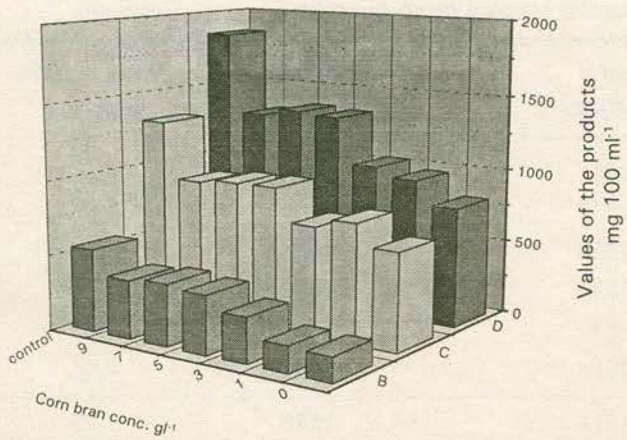
0, control; amm sulphate, 2g l^{-1}; incubation period, 4 days at 32°C; medium (g l^{-1}; wv^{-1}): molasses, 140; amm sulphate, different levels; $CaCO_3$, 1.5; rice bran, 5; corn bran, 5; at pH 5.5.

Fig 5. Effect of different concentrations of dibasic amm phosphate on acetone butanol production by *C. acetobutylicum.*



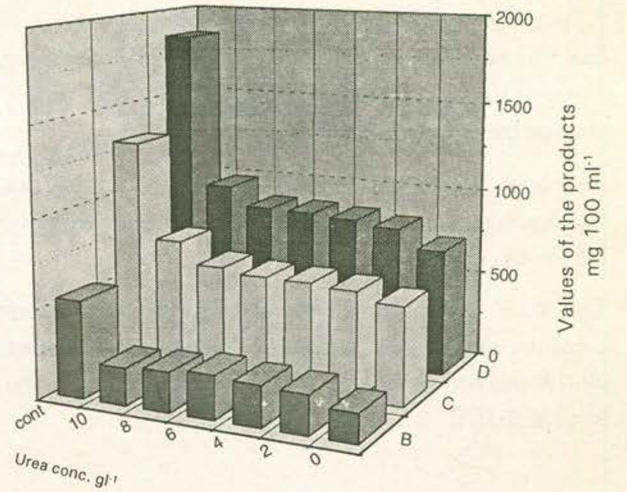
Control, amm sulphate, 2g l^{-1}; incubation period, 4 days at 32°C; medium (g l^{-1}; wv^{-1}): molasses, 140; pot. nitrate, different levels; $CaCO_3$, 1.5; rice bran, 5; corn bran, 5; at pH 5.5.

Fig 6. Effect of different concentrations of pot. nitratre on acetone butanol production by *C. acetobutylicum.*



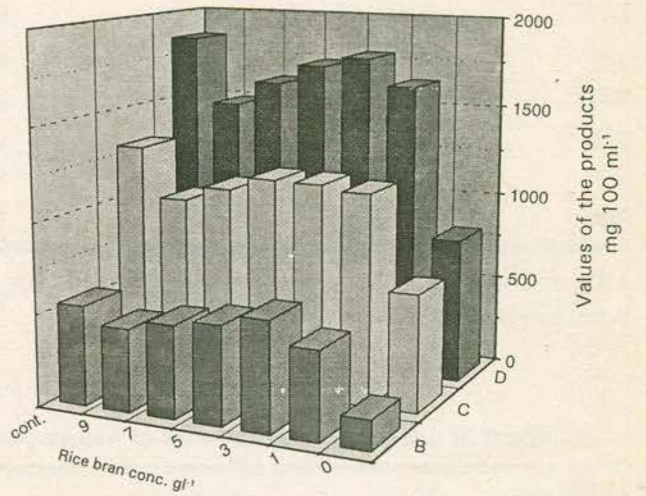
Control, rice bran, corn bran (5 g l^{-1} for each); incubation period, 4 days at 32°C; medium (gl^{-1}; wv^{-1}): molasses, 140; amm. sulphate, 2; $CaCO_3$, 1; urea, different levels.

Fig 7. Effect of different concentrations of urea on acetone butanol production by *C. acetobutylicum.*



Control, rice bran corn bran (5 g l^{-1} for each); incubation period, 4 days at 32°C; medium (g l^{-1}; wv^{-1}): molasses, 140; amm. sulphate, 2; $CaCO_3$, 1; rice bran, different levels.

Fig 8. Effect of different concentrations of rice bran on acetone butanol production by *C. acetobutylicum.*



Control, rice bran corn bran (5 g l^{-1} for each); incubation period, 4 days at 32°C; medium (g l^{-1}; wv^{-1}): molasses, 140; amm. sulphate, 2; $CaCO_3$, 1; corn bran, different levels; B, value of acetone; C, value of butanol; D, value of total solvent.

Fig 9. Effect of different concentrations of corn bran on acetone butanol production by *C. acetobutylicum.*

acetone butanol production among the tested inorganic nitrogen sources. The results were in accordance with that of Spivey (Spivey 1978) who used $(NH_4)_2SO_4$ as nitrogen source at 2g l^{-1}. Other workers (Petitdemange *et al* 1976; Gattschal and Morris 1981) used synthetic media containing 2 to 3 g l^{-1} $(NH_4)_2SO_4$ as the nitrogen source.

Effect of the nature of the organic nitrogen source: Urea. The results (Fig 7) indicated that urea was unsuitable organic nitrogen source for solvents production, since it sharply decreased the amount of both solvents.

Rice bran. The results represented in Fig 8 revealed that the maximum solvent yield (17.89 g l^{-1}) was obtained by using 0.3g% of rice bran.

Corn bran. The results (Fig 9) showed that, as the amount of corn bran was increased the amount of total solvents increased unitl it reached its optimum value (13.58 g l^{-1}) at corn bran level of 0.7 g%.

Rice bran and corn bran in mixture. The results (Table 2) showed that the solvent production was clearly affected by the different concentrations of rice bran and corn bran. The maximal output of solvents (18.56) was recorded at 5 g l^{-1} for both.

Fodder yeast. The results shown in Table 3 revealed that the solvent yield increased with the increase of fodder yeast concentration reaching its optimal value at 2 g l^{-1}, above which a gradual decrease of solvent yield was obtained.

Yeast autolyzate. The results (Table 4) showed that the maximum yield of solvents (18.56 g l^{-1}) was obtained by using

Table 2

Effect of different concentrations of rice bran and corn bran on acetone butanol production by *C. acetobutylicum*

Rice bran / corn bran (g l^{-1})	Final pH	Acetone (mg100ml^{-1})	Butanol (mg100ml^{-1})	Total solvents (mg 100ml^{-1})	Residual sugars (gm 100ml^{-1})
0	5.33	162	644	806	4.5
1/1	4.89	437	904	1341	3.2
3/3	4.85	472	1101	1573	2.5
5/5	4.86	545	1311	1856	1.35
7/7	4.85	510	1215	1725	1.67
9/9	4.91	511	1189	1700	1.75

1. The medium composition (g l^{-1} wv^{-1}): molasses, 140; $(NH_4)SO_4$, 2; $CaCO_3$, 1; rice bran, different levels; corn bran, different levels at pH 5.5
2. Incubation period, 4 days at 32°C.

Table 3

Effect of different concentrations of fodder yeast on acetone butanol production by *C. acetobutylicum*

Fodder yeast (g l^{-1})	Final pH	Acetone (mg100ml^{-1})	Butanol (mg100ml^{-1})	Total solvent (mg 100ml^{-1})	Residual sugars (g 100ml^{-1})
0	5.33	162	644	806	4.5
2	4.75	474	1235	1709	1.86
4	4.84	454	1230	1684	2.94
6	4.80	453	1230	1683	2.94
8	4.72	296	853	1149	3.57
10	4.80	269	711	980	4.46
Control	4.86	545	1311	1856	1.35

1. The medium composition (g l^{-1} wv^{-1}); molasses, 140; $(NH_4)_2SO_4$, 2; $CaCO_3$, 1; fodder yeast, different levels at pH 5.5
2. Incubation period, 4 days at 32°C,
3. Control, using rice bran and corn bran (5 g l^{-1} each).

Table 4

Effect of different concentrations of yeast autolyzate on acetone butanol production by *C. acetobutylicum*

Yeast autolyzate (g l^{-1})	Final pH	Acetone (mg100ml^{-1})	Butanol (mg100ml^{-1})	Total solvents (mg 100ml^{-1})	Residual sugars (g100ml^{-1})
0	5.33	162	644	806	4.5
2	4.52	159	655	814	4.48
4	4.84	522	1095	1617	2.29
6	4.85	545	1311	1856	1.35
8	4.84	521	1321	1842	1.41
10	4.84	515	1288	1803	1.45
Control	4.86	545	1311	1856	1.35

1. The medium composition (g l^{-1} wv^{-1}): molasses, 140; $(NH_4)_2SO_4$, 2; $CaCO_3$, 1; yeast autolyzate, different levels at pH 5.5
2. Incubation period, 4 days at 32°C,
3. Control, using rice bran and corn bran (5 gl^{-1} each).

6 g l^{-1} yeast autolyzate. The use of lower or higher levels of yeast autolyzate gave inferior yields of solvents.

Therefore, among the organic nitrogen sources tested, a mixture of rice bran and corn bran 5 g l^{-1} each was the best. Also the same solvent yield was obtained 18.56 g l^{-1} on using 6 g l^{-1} yeast autolyzate. However, rice bran and corn bran mixture was preferably used as both sources were cheaper and easily available. Rice bran and corn bran are suitable organic nitrogen sources because of their versatile contents of many biosis such as amino acids, vitamins and essential elements. Also, rice bran and corn bran proved to be the best and cheapest food by product as sources of organic nitrogen. The same author found that 5 g l^{-1} rice bran and 8 g l^{-1} corn bran were optimal for solvent production by *C. acetobutylicum*.

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