PRODUCTION OF ACETONE BY LOCAL STRAINS OF ANAEROBIC SPOREFORMERS

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INTRODUCTION

It is well known that there are numerous species of soil-inhabiting anaerobic sporeformers which ferment carbohydrates with the production of acetone-butanol. These solvents are of enormous value in drugs, paints, synthetic rubber, explosives, plastics ... etc.

The production of acetone and butanol by fermentation started on a commercial scale at the beginning of the World War I. After the World War II, the synthetic method replaced gradually the fermentation method in many countries, in which the production of petro-chemicals has been developed. Nevertheless, in many other countries where agricultural and industrial waste-products are available and economical, the production of acetone and butanol by fermentation is still carried out.

In view of the fact that many waste products in Egypt such as corn cobs, inedible grains, potatoes, sweet potatoes and molasses are potential sources for raw materials, hence the production of acetone-butanol by fermentation could be considered economical.

The aim of this work is to investigate the following points :

1- Isolation of local strains of great fermentative activities in the production of acetone-butanol.

2- Several available raw materials such as corn, millet, corn cobs, saw dust, sweet potato and molasses were investigated as carbon sources. Other by-products such as brewery yeast, peanut cake, cotton seed cake, rice bran and corn steep liquor were investigated as nitrogen sources.

3- The study was also extended to cover other nutritional and invironmental factors that influence the production of acetone-butanol.

The inter-play of these studies may lead to the best nutritional and invironmental conditions that give the best economical yield of acetone-butanol.

REVIEW OF LITERATURE

Historical :

Historical development of acetone-butanol fermentation has already been described in reviews, such as those of Prescott and Dunn (1959). They stated that normal butyl alcohol was discovered as a regularly occurring constituent of fusel oil by Wurtz in 1852. Pasteur, however, was the first investigator to show that butyl alcohol was a direct product of fermentation of lactic acid and calcium lactate.

The need for synthetic rubber resulted in the first successful commercial process, which was established in England by the Firm of Strange and Graham Ltd., during the year 1913 and 1914. Significant research was carried out by this firm which employed Perkin, Weizmann, and others. Weizmann left the employ of this firm in 1912 and not long afterward isolated a starch-fermenting anaerobic organism, which isolate later became known as Clostridium acetobutylicum weizmann and was used in industrial processes (Prescott and Dunn, 1959).

Soon thereafter plants were placed in operation in Canada, the United States, and India. It was the acetone which was of primary concern at this time, because of its use in the making of explosives and for other purposes. With the termination of World War I, the plants were closed because acetone was no longer needed in large quantities. It was not long, however, before a demand arose for n-butanol in the manufacture of automobile lacquers (Prescott and Dunn, 1959).

During the year 1919 several papers concerning the acetone and aceton-butanol fermentations were published. Nathan described the manufacture of acetone by the Weismann process from raw materials as corn and horse chestnuts. Butanol and acetone attained a high level during the Second World War (Prescott and Dunn, 1959).

Nowadays, the synthetic method replaced gradually the fermentation process, but the apportunity still exists for the latter process, to be carried on, in countries, where agricultural and industrial wastes are present and can be processed economically.

Organisms producing acetone and butanol :

A number of species of microorganisms produce acetone and butanol, but only a few do so in significant quantities, (Table 1). Species which are noted for this ability include those belonging to the genera Bacillus and Clostridium, as reported by many investigators as Weizmann and Rosenfeld (1937), Paul (1963) and Abd El-Samie (1971).

McCoy et al. (1930) have classified the motile and sporeforming butyric anaerobes of fermentation as a non-pathogenic subgroup of the genus Clostridium, which yields larger amounts of butyric acid or neutral products than the pathogenic clostridia, gives the granulose reaction, and is catalase negative. The butyric anaerobes were divided into two general groups :

Group 1. Acid end products, chiefly butyric and acetic acids - the true butyric anaerobes.

Subtype A. Cl. pasteurianum type-non starch-fermenting bacteria.

Subtype B. Bacillus saccharobutyricus type-

Table 1 : Comparative studies between several isolates producing aceton and butanol

Worker	Substrate used	Organisms used	T.N.V.P* % based on the weight of raw material	T.N.V.P* % based on the weight of sugar
Saji (1933)	Millet	*B. granulobacter pectinovorum*	23.9	
Rokusho (1936)	Rice	*Cl. acetobutylicum*	25.9	
Zykova (1939)	Cereals	*B. acetoaethylicus*		42
Baba (1943)	Cane molasses	*Cl. toanum*		35
Weizmann (1945)	Black strap molasses	*Cl. acetobutylicum*	28.8	
Makhmanovich *et al.* (1963)	Corn cobs	*Cl. butyricum prazmowsky*		30 - 38
Paul (1963)	Sweet potato	*Cl. acetobutylicum.*	28 - 34	
Abd El-Samie (1971)	Corn	*Cl. acetobutylicum*	22.6 - 24.6	

* T.N.V.P % = Percentage of total neutral volatile products.

starch-fermenting clostridia and occasionally plectridia.

Subtype C. Starch-fermenting plectridia.

Group 2. Butyric and acetic acids as intermediate products, followed by neutral products (alcohols, or alcohol plus acetone) - the butanol organisms of industry.

Acetone bacteria were isolated from different sources, i.e. soils cultivated with different crops, corn, stalks, rotted wood and river mud. (Muta, 1933; Legg and Stiles, 1935).

Beesch (1952) and McCutchen and Hickey (1954) recommended the use of potato glucose mash for the isolation of the saccharolytic types, on account of the favourable effect of starch, when present together with glucose, on the spore germination. The latter authors recommended also barley mash (10 %) as the initial isolation medium for both the saccharolytic and starch fermenting types. However, quantitative analysis on fully fermented cultures, will discriminate between the desired and undesired ones according to their capacity of solvents production. Promising cultures are plated out on malt gelatin agar (Weyer and Rettger, 1927) under anaerobic conditions.

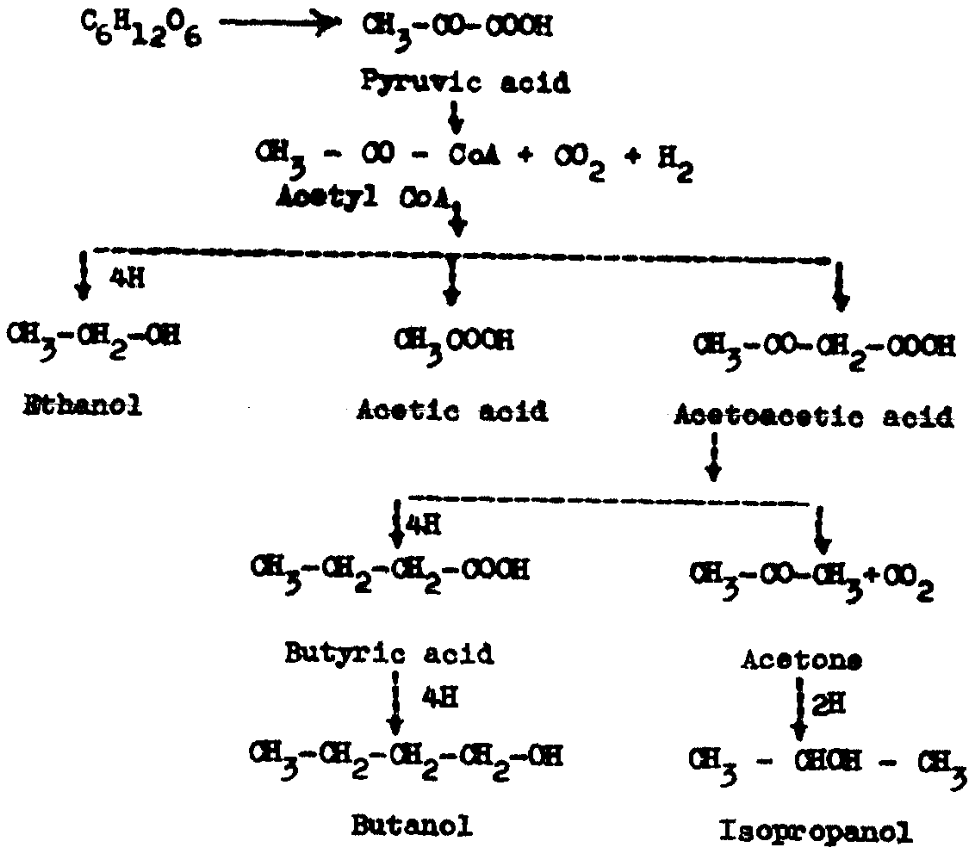
Typical colonies are picked, and examined quantitatively for solvents production from typical fermentation mashs i.e. corn mash, molasses, corn cobs hydrolysates...etc.

Scheme of fermentation :

Several schemes have been suggested to explain the origin of the end products of a normal butanol-acetone fermentation.

Speakman (1923) was one of the first investigators who suggested a detailed scheme for the mechanism of the butanol-acetone fermentation. He explained that, butyric and acetic acids were formed by cleavages and oxidations of the sugar molecule, then these acids were reduced in part to the corresponding solvents.

Salle (1961) stated that in this type of carbohydrate breakdown, the two chief end-products are butyl alcohol and acetone. Other products include ethyl alcohol, isopropyl alcohol, butyric and acetic acids, hydrogen and carbon dioxide. The scheme for the fermentation of glucose by *Cl. acetobutylicum* is given below :



Wood <u>et</u> <u>al</u>. (1944) used heavy carbon (C^{13}) compounds as tracers in an attempt to determine the mechanism of the fermentation. When acetic acid $CH_3 - {}^{*}C^{13}OOH$ was added to the fermentation, butyl alcohol was found which contained heavy carbon. Butyric acid $CH_3 - {}^{*}C^{13}H_2 - CH_2 - COOH$, was isolated which contained heavy carbon in the carboxyl and Beta positions. On the