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A LA MEMOIRE D'ATATÜRK AU CENTENAIRE DE SA NAISSANCE



**The Biological Degradation of Pure Pectin And Citrus
Wastes To Obtain Fuel Gas**

by

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DEDICATION TO ATATÜRK'S CENTENNIAL

Holding the torch that was lit by Atatürk in the hope of advancing our Country to a modern level of civilization, we celebrate the one hundredth anniversary of his birth. We know that we can only achieve this level in the fields of science and technology that are the wealth of humanity by being productive and creative. As we thus proceed, we are conscious that, in the words of Atatürk, "the truest guide" is knowledge and science.

As members of the Faculty of Science at the University of Ankara, we are making every effort to carry out scientific research, as well as to educate and train technicians, scientists, and graduates at every level. As long as we keep in our minds what Atatürk created for his Country, we can never be satisfied with what we have been able to achieve. Yet, the longing for truth, beauty, and a sense of responsibility toward our fellow human beings that he kindled within us gives us strength to strive for even more basic and meaningful service in the future.

From this year forward, we wish and aspire toward surpassing our past efforts, and with each coming year, to serve in greater measure the field of universal science and our own nation.

The Biological Degradation of Pure Pectin And Citrus Wastes To Obtain Fuel Gas

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SUMMARY

The production of valuable goods or usable materials from various wastes and the protection of the environment from the pollution are the important problems of this century. For this purpose, all sorts of wastes with different characters and too many methods are used.

Agricultural wastes have an important place amongst others. One of the method used is fermentation, especially anaerobic fermentation which in most cases helps to solve energy problem.

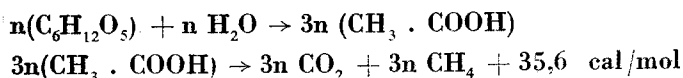
In the following article the anaerobic fermentation of citrus wastes and pectin are investigated. According to the results obtained, the gas produced from anaerobic disintegration of the mentioned wastes contains approximately 50 % of hydrogen gas which can be used either as a fuel or as a chemical in process industries.

INTRODUCTION

One of important, probably the most important problem that our civilisation faces; is no doubt the shortage of the energy. The limited amount of fossile fuels and the big increase on crude petrol prices force the researchers either to establish a suitable and economical method to store the solar energy or to find out new energy sources, amongst them is the "Biogas" which can be produced by the utilisation of the agricultural wastes and/or of the manure.

It is a very well known fact that fresh manure is not suitable to be used as natural fertilizer. For the destruction of the wild seeds which are present in it, for the elimination of pathogenic microorganisms and for the increase of C/H ratio from 1/30 to 1/20 which is achieved by the loss of water and carbon dioxide through aerobic degradation; the manure should be let to stand for a couple of months in an open dung-

hill. During this period a great deal of the valuable chemicals e. g carbon, nitrogen, potassium and phosphorus are lost. Hence 30 % of the original dry material is wasted. These losses can be prevented successfully if the anaerobic fermentation process is preferred. The anaerobic degradation of the cellulosic materials takes place in the following two basic steps:



These reactions do not of course indicate the stoichiometric relations in an anaerobic disintegration process, they only point to the overall mechanism of the fermentation.

The raw material, manure in general, which is rich in carbohydrates (17 to 25 % cellulose) and contains necessary nutrients for the bacterial activities, can be fermented anaerobically that produces carbon dioxide and methane according to the mechanism given. After being diluted to a definite concentration (10 to 12 % dry material) the manure can be anaerobically disintegrated in a closed and sealed vessel at 20 to 35°C degrees or at higher temperatures¹. When the oxygen which is present in free volume of the digester, is purged out by the carbon dioxide that produced by the aerobic fermentation of the mixture, the anaerobic fermentation starts to proceed. 100 kg of dung can produce approximately 5 cu m of gas which is named as "Biogas" and is consisted essentially of methane and carbon dioxide. This gas mixture can be collected and stored in a suitable gasometer readily available for use whenever required. The fermented slurry is a high quality natural fertilizer that can easily be handled.

Fermenting the manure anaerobically is a beneficial process which can secure the following objectives:

a- A high calorific value gas is produced. The gross calorific value of the biogas is 5902 k. cal per cu m and it can substitute city gas.

b- After being purified and compressed in steel bottles it can be used as fuel in gas engines. 1 cu m of biogas is equivalent to 1,44 cu m of city gas, 0.75 liter of gasoline or 0.55 kg of diesel oil.

c- From the hygienic point of view, collecting the manure in a closed and sealed container prevents the dispersion of the different types of bacteria into the atmosphere and the environment to secure the human and the animal population from the contagious diseases.

d- A great deal of time is saved to adjust the C/H ratio and in the mean time valuable plant nutrient chemicals are regained.

The number of the cattles in this Country is 15.5×10^6 (²). If the amount of excrement from a single animal is 10 kg per day, the yearly production of manure will sum up to 5.66×10^7 tons. Remembering that 1 ton of manure is equivalent to 50 cu m of biogas, the corresponding amount of gas will be 2.26×10^9 cu m per year. If it is taken in consideration that 1 cu m of the gas can replace 0.75 liter of gasoline or 0.55 kg of diesel oil, from the point of view of the energy content, 2.26×10^9 cu m of gas will be equal to 1.58×10^9 liters of gasoline or 1.24×10^9 kg of diesel oil per year.

As the increase of the productivity of the soil by using the fermented slurry as natural fertilizer, is out of the scope of this work, it is not taken into consideration in this study.

The development on citrus growing and related industries have given birth to an important environmental problem; that is the destruction of the peels. Although they can be used to feed animals, their carbohydrate content has given the idea to use them as raw material in anaerobic fermentation process to produce a fuel gas similar to that of biogas.

All citrus fruits have continuous layer of epidermal cells with a thick cuticle containing stomata. Under this epidermis lies the epicarp or flavedo. Penetrating into the peel of citrus fruit, one comes to the white, spongy inner rind generally known as the albedo which is the main concern of this work.

The albedo portion of the fruit represents about 20 to 60 % of the whole fruit. The thickness of the albedo varies according to the different varieties of the citrus fruits. The thickness of the albedo of *Citrus sinensis* (Orange), *Citrus paradisi* (Grape Fruit) and *Citrus luminciana* (Etrog) ranges from 3 to 5 mm, 8 to 12 mm and 15 to 20 mm respectively.

Fresh albedo contains about 75 to 80 % of water while its main components calculated on dry basis consist roughly of 44 % of sugars (in a ripe fruit), 33 % of cellulose (including lignin and pentosans) and 20 % of pectinous substances. A complete analysis of *Citrus paradisi* has been given by Poore³.

In the plants where citrus fruit is fully utilised all of these find their applications. Citrus peel as a source of sugar, can of course, be sub-

jected to alcoholic fermentation process and as well as to the other types of processes such as for instance acetic acid or butanol-acetone fermentations.

Acetic acid fermentation process is used commercially in the production of vinegar. Butanol-acetone fermentation was applied to disintegrated and essential oil extracted peels and it was shown that during the butanol-acetone fermentation the pectin of the albedo was hydrolysed and partially demethylated; more than 500 cu m of gas (45 % of hydrogen, 55 % of carbon dioxide) was evolved during the fermentation of one ton of sugar contained in the peel⁴.

Another important component of the citrus peel is pectin which is first discovered by Braconnot in 1825 as the "gelatinous principle of fruits" and since then an extensive research work has been done to throw light on every aspects of this physiologically as well as commercially important substance. From the commercial point of view pectin is the most important component of albedo, for commercial citrus pectin is now being extensively produced in powder form and is used in large quantities in the manufacturing of jams and many other products such as emulsifying, rubber latex creaming, steel hardening agents.

As it has already been mentioned above that the aerobic butanol-acetone fermentation of the peel produces hydrogen and carbon dioxide gases. If the presence of the methylated carboxyles are taken in consideration one can find reasonable to expect methane gas from the anaerobic fermentation process of the albedo, remembering the anaerobic disintegration mechanism. This theoretical assumption has stimulated this work.

Experimental

1- Material: *Citrus sinensis* and *Citrus paradisi* were purchased from the market. *Citrus limonciana* was obtained from the Collection Gardens of the Ministry of Agriculture in Dörtüol near Adana.

Fresh fruits were cut into halves and using a spoonlike device the pulps (juicy part of the whole fruit) were removed. The coloured part, known as flavedo was shaved off with a sharp knife. The remaining spongy, white mass, named as albedo, was dried in a vacuum oven at 80°C and 350 mm Hg pressure for 24 hours. Dry albedo was ground and sieved to 16 mesh (1,00 mm) and used for the fermentation studies.

Pure pectin required for the fermentation studies was extracted in two steps from dried albedo. From its water solutions pectin is rea-

dily precipitated by alcohol or acetone as suspended jelly which in turn is again soluble in water.

100 g of dry and ground to pass an ASTM 100 mesh sieve (0.15 mm) albedo was made into a slurry with 1.5 l of distilled water in a two liter beaker. Stirring vigorously 35.5 % of hydrochloric acid was added into the slurry until the pH was brought down to about 1.9 to 2.0. The Lyphan Universal indicator papers were used to check the pH. The mixture was let to stand in a thermostated water bath at 70°C for one hour. The acidified slurry was than transferred to six centrifuge tubes, 250 ml each. After being centrifuged for 15 minutes at 1500 rev/min the viscous pectin solution was separated from the slurry. The solid material was subjected to second extraction as described above. In the second extraction the amount of distilled water used was 550 ml. At the end of two subsequent extractions 1.5 l of viscous solution of pectin was obtained. This solution was then poured into 96 % alcohol to precipitate pectin. The required amount of alcohol for the precipitation was calculated in such a way that its final dilution after the operation is to be 60 %. The jelly like solid thus precipitated was filtered by a cloth sac filter and washed with alcohol until no chloride reaction was observed. The acid free pectin so obtained was then pressed to remove the excess of alcohol and dried in a vacuum oven at 80°C degrees and 350 mm Hg pressure for 24 hours. Table 1 shows the pectin yields of the albedos of different citrus varieties and the analysis of the pectin produced.

Table-I: Pectin Yields of Different Citrus Varieties and the Analysis of the Pectin Produced

| Citrus Species | Pectin Yield % | Dry Pectin % | Water % | Ash % | Methoxyl % |
|-------------------|----------------|--------------|---------|-------|------------|
| Citrus paradisi | 17.5 | 87.6 | 10.9 | 1.5 | 6.7 |
| Citrus sinensis | 23.5 | 89.9 | 8.16 | 1.9 | 7.9 |
| Citrus luminciana | 16.7 | 88.3 | 10.43 | 1.3 | 5.3 |

Considering the difficulties in obtaining and transporting of the isolated pure anaerobic bacteria, inoculant was prepared simply and cheaply from the content of the cattle stomach. Just after slaughtering the animal, 1 % of the content of the stomach was added to the diluted (10 %) manure. The air was purged out from the free volume of the digester by carbon dioxide and the mixture was left for the fer-

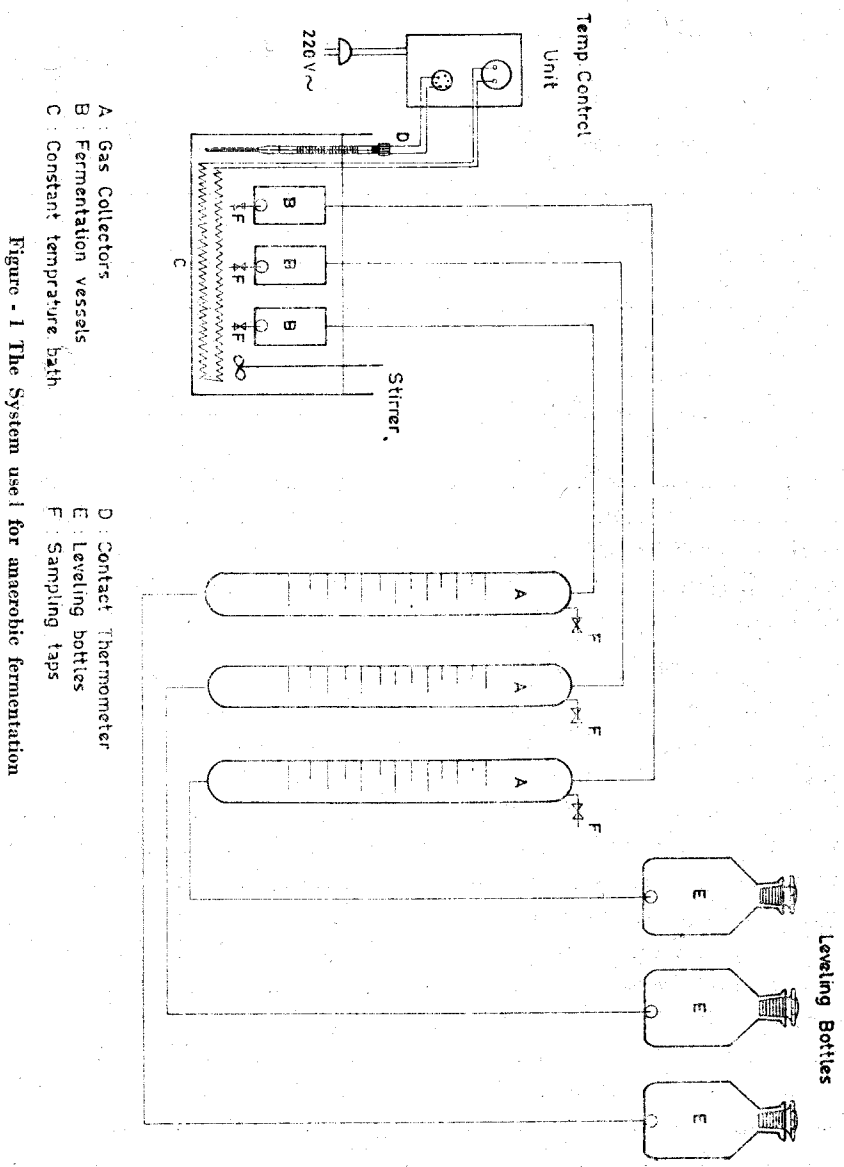
mentation to occur at 35°C degrees. The produced gas from the reactor was collected in a cylinder and analysed daily to follow the methane percentage in the mixture. When it was reached to its peak value the substrate was used to inoculate the albedo mixtures for anaerobic fermentations.

2- Method: The system used for experimental works is shown in Figure 1. It consists of (a) a thermostated water bath furnished with an electrical heater, a contact thermometer and a mechanical stirrer, (b) required number of digestors with 2.0 liters of working volume, (c) graduated measuring cylinders, specially made for the purpose to collect and to measure the volume of the gas produced, (d) and leveling bottles.

50 g of dry (90 % dry material) and ground (16 mesh) albedo was put in a digester and was slurried with 1200 ml of distilled water. The pH of the mixture was adjusted with 5 N potassium hydroxide, hence required potassium as nutrient for the microorganisms was also provided. After having the pH adjusted the substrate was inoculated 200 g of already fermented manure. The digester then was situated in water bath. To purge out the oxygen, carbon dioxide was used. For the fermentation of the albedos of the three citrus varieties no nitrogen addition was required as the nitrogen contents of albedos were ranging from 1.0 to 1.5 %. See Table -II. But in the case of anaerobic fermentation of pure pectin the quantity of nitrogen supplied by inoculant was not sufficient. Therefore to get the anaerobic fermentation of pure pectin started 200 mg per liter of nitrogen was added as 1 % of ammonia solution. After having all these preparations completed the digester was connected to the measuring cylinder and the substrate was let to ferment at $35^{\circ} \pm 1$ C degrees. To destroy floating layer on top of the liquid phase and to increase the contact surface area between microorganisms and the substrate, the fermentors were shaken at least three or four times in a day.

Results and Discussion

As it can be seen from the Table-II, the general character of the fermentation of the albedo of the three citrus species were very similar. The degradation was started in 7 to 8 hours and was lasted for five days. The highest volume of gas was obtained in the first 24 hours. To get the highest volume of gas in the anaerobic fermentation of pure pectin two days were necessary (Fig. 2). The evolution of gas decreased



- A : Gas Collectors
- B : Fermentation vessels
- C : Constant temperature bath
- D : Contact Thermometer
- E : Leveling bottles
- F : Sampling taps

Figure - 1 The System used for anaerobic fermentation

Table-II: The Data Obtained in Anaerobic Fermentation of the Albedo and Pure Pectin

| Sample | Dry mater. loss % | Gas produc. per 100 g of dry mater. Liter (NTP) | Dry material in substrate | | pH of the substrate | | Gas prod. per 100 g of disint. dry mate. Liter (NTP) | Dry mater. in albedo % | Nitro. gen % |
|-------------------|-------------------|---|---------------------------|-------------|---------------------|-------------|--|------------------------|--------------|
| | | | Before Ferm. | After Ferm. | Before Ferm. | After Ferm. | | | |
| Citrus paradisi | 2.00 | 8.25 | 5.25 | 3.25 | 7.0 | 5.5 | 21.60 | 92.29 | 1.1 |
| Cit. sinensis | 2.07 | 8.35 | 5.23 | 3.16 | 7.0 | 5.0 | 21.35 | 91.57 | 1.5 |
| Citrus luminciana | 2.09 | 8.35 | 5.25 | 3.16 | 7.0 | 5.5 | 21.22 | 91.74 | 1.2 |
| Pectin | 1.51 | 7.3 | 4.25 | 2.74 | 7.2 | 5.8 | 17.70 | 99.00 | - |

gradually until the degradation was stopped completely. During the fermentation period the amount of the gas produced by the different varieties of citrus albedo was the same. This observation can be taken as the designation of the same albedo composition that extracted from different citrus species.

The pectins are composed mostly of polygalacturonic acids of different degrees of esterification and neutralisation and show wide variations in their solubility in water⁵. Pectins derived from various sources differ on account of proportions of free acid groups which have been neutralised to form salts. A further cause of variation is the size and the the distribution pattern of polygalacturonic acid chains. During the preparation a fractionation typical of the method used occurs; the chemical and colloidal properties of pectins will therefore, vary greatly with different methods of preparation.^{6,7} The examination of the Table-I shows that; the properties of the pectins from different albedos are very similar. Therefore the pure pectin isolated from each variety was not fermented separately, instead a blend of them was used. The induction period of the fermentation of pure pectin was again 7 to 8 hours. The duration of the disintegration of pure pectin was two days shorter comparing to the degradation of albedo. The relatively shorter period of fermentation can be attributed to the lack of polysaccharide impurities. It can easily be assumed that under the choosen conditions of fermentation no selective degradation is to be expected, therefore not only pectins but also other carbohydrate components of the albedo were to

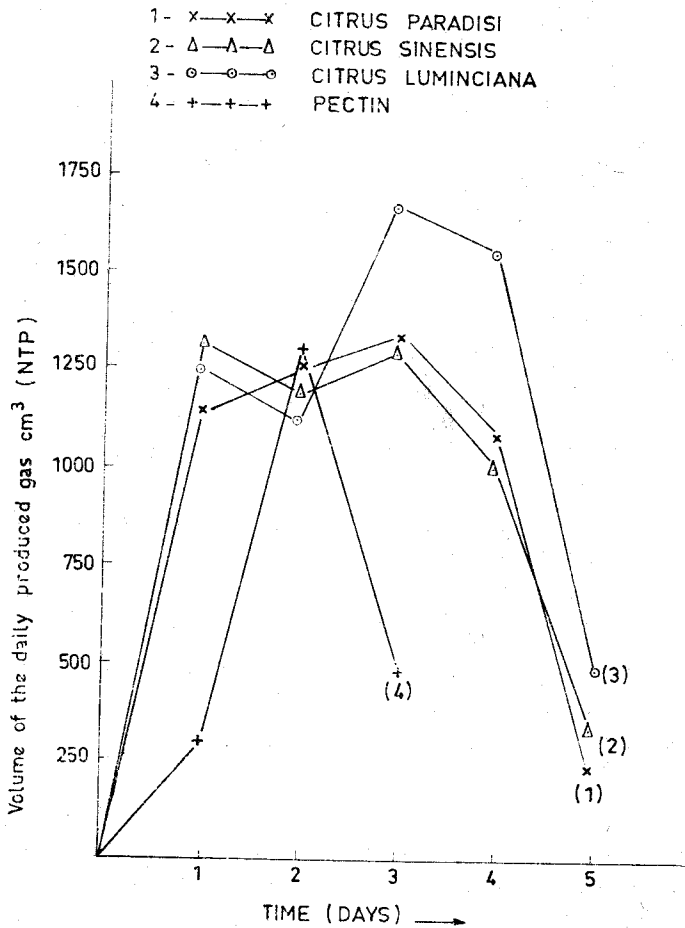


Figure 12: Daily gas production

be disintegrated. Thus degradation of the albedo lasts longer than the pure pectin does.

Another point which attracts the attention is the induction period of the process. Either albedo or pure pectin start to ferment in about 7 to 8 hours while the dung does in 10 or 12 days. This behaviour is most probably related to the strength of the β linkage between glucose units in cellulose. Although the linkage between galacturonic acid units is similar to that in cellulose, the pectin molecule is not resistant to alkalis⁸. In alkaline solutions they can easily be demethylated and the molecular weight decreases to the one third of its original value. Thus the first stage of the degradation is simply achieved just making the mix-

ture alkaline. On the other hand, in cellulose, the first stage of the fermentation proceeds enzymatically and takes longer times comparing to the fermentation of pectin.

The conventional solid loading in anaerobic processes is 10 to 12%⁹. But 10 % of dry material in the substrate did not produce any gas in the anaerobic degradation of pectin. However reasonable amount of gas was only obtained when the solid content of the substrate was reduced to 5 %. It was thought that the necessity of the low substrate concentration was due to the high carboxyle group content of the pectin. In general microorganisms are not effected from hydrogen ions. But weak acids e.g acetic acid, are not completely dissociated in their water solutions. Keeping their molecular structure in solutions renders these acids easily penetrable trough the mebrane of the cell. Once in they alter the composition of the protoplasma which in turn stops the biological activities of the cell¹⁰. The acetic acid content of the pectin ranges from 10 to 12.5 % and the disintegration of it also produces the same acid¹¹. Under these circumstances it is reasonable to relate the ceasing of the fermentation, to the presence of acetic acid. In fact before the inoculation, the pH of the substrate has been risen to 9, but in 2 to 3 hours time, due to the neutralisation, the pH dropped to 7.0 .As the pH of the substrate in anaerobic disintegration processes is either neutral or slightly alkaline no further alkali was added. When the evolution of gas has stopped, the pH value of the substrate was 5.0 i.e the acid was predominating. Increasing the pH of the substrate again to neutral, helps the fermentation to take place for another 24 hours and it again ceases on account of the decrease of pH to 5.0. As long as the acid predominates there is always enough acetic acid in molecular structure to penetrate through the membrane of the cell to hinder biological activities which in turn causes to stop or at least to retard the degradation. It seems reasonable to conclude that, when the pH decreases to 5 the limit of the concentration of acetic acid in substrate is exceeded¹².

The anaerobic fermentation of the albedos of *Citrus sinensis*, *paradisi* and *luminciana* produced 21.0 liter (NTP) of gas per 100 g of disintegrated dry material (Table-II). The amount of gas evolved from the fermentation of pure pectin was 17.7 liter (NTP). This difference comes of course from the organic impurities present in albedo. They too, were disintegrated and have increased the volume of the gas obtained.

The product gas was analysed with an explosion pipette included Orsat apparatus. The results are summarised in Table-III and are shown

Table-III: The Daily Analysis of the Produced Gas Mixture

| Citrus Species | First Day | | Second Day | | Third Day | | Fourth Day | | Fifth Day | |
|-------------------|-------------------|------------------|-------------------|------------------|-------------------|------------------|-------------------|------------------|-------------------|------------------|
| | CO ₂ % | H ₂ % | CO ₂ % | H ₂ % | CO ₂ % | H ₂ % | CO ₂ % | H ₂ % | CO ₂ % | H ₂ % |
| Citrus paradisi | 75 | 25 | 72 | 28 | 55.5 | 44.5 | 52 | 48 | 51 | 49 |
| Citrus sinensis | 72 | 28 | 68 | 32 | 66 | 34 | 59 | 41 | 57 | 43 |
| Citrus luminciana | 68 | 32 | 61 | 39 | 57.5 | 42.5 | 58 | 42 | 58 | 42 |
| Pectin | 82 | 18 | 63 | 37 | 55 | 45 | --- | --- | --- | --- |

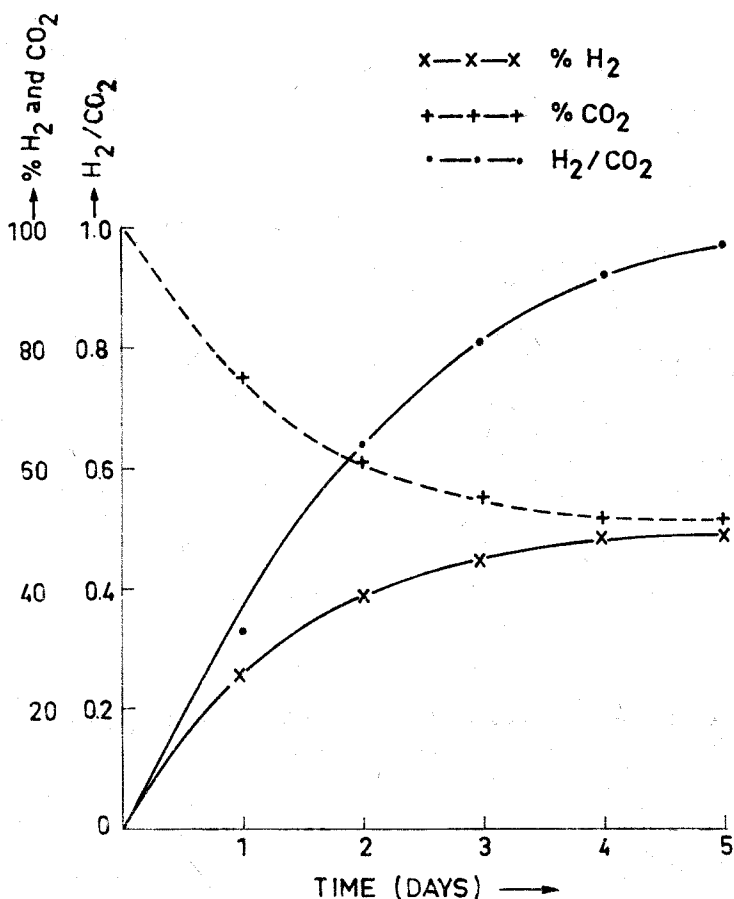


Figure - 3: The daily variations of the CO₂, H₂ percentage of the gas produced from citrus paradisi and the H₂/CO₂.

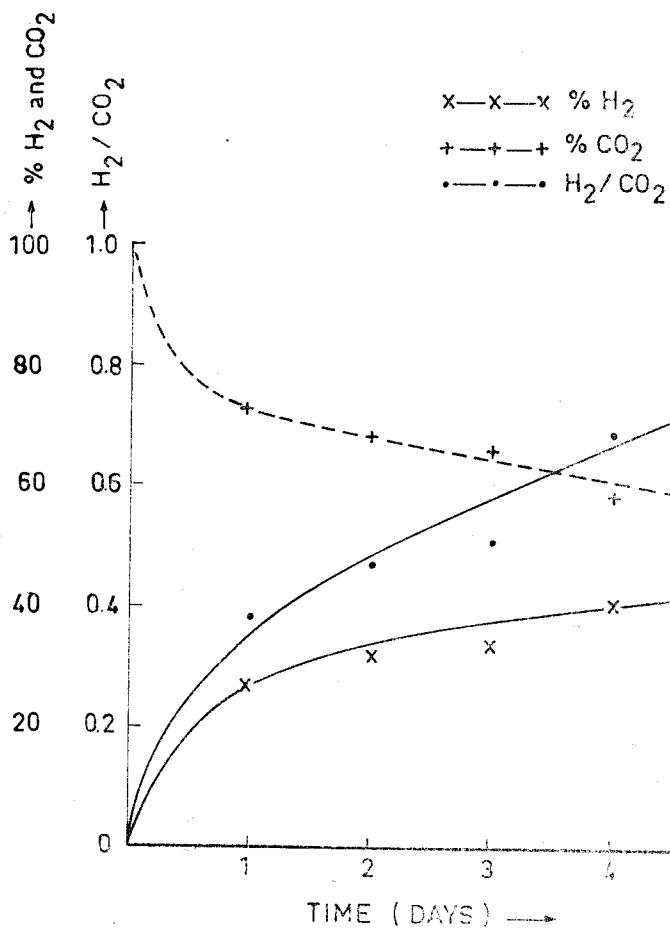


Figure - 4: The daily variations of the CO₂, H₂ percentage of the gas produced from citrus sinensis and the H₂/CO₂.

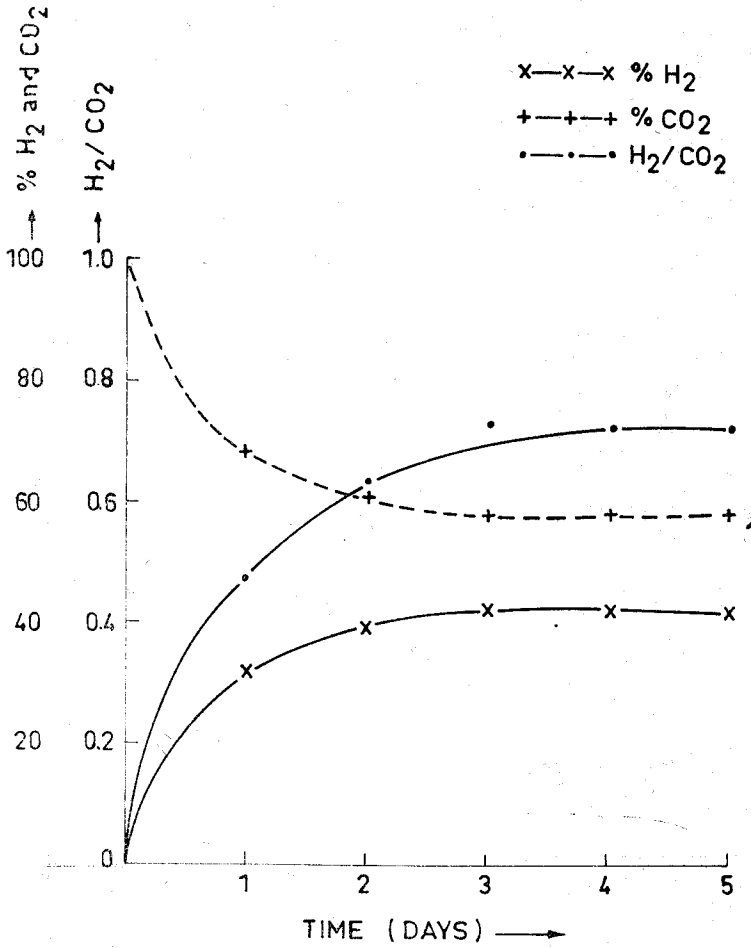


Figure - 5 The daily variations of the CO₂, H₂ percentage of the gas produced from citrus luminciana and the H₂/CO₂.

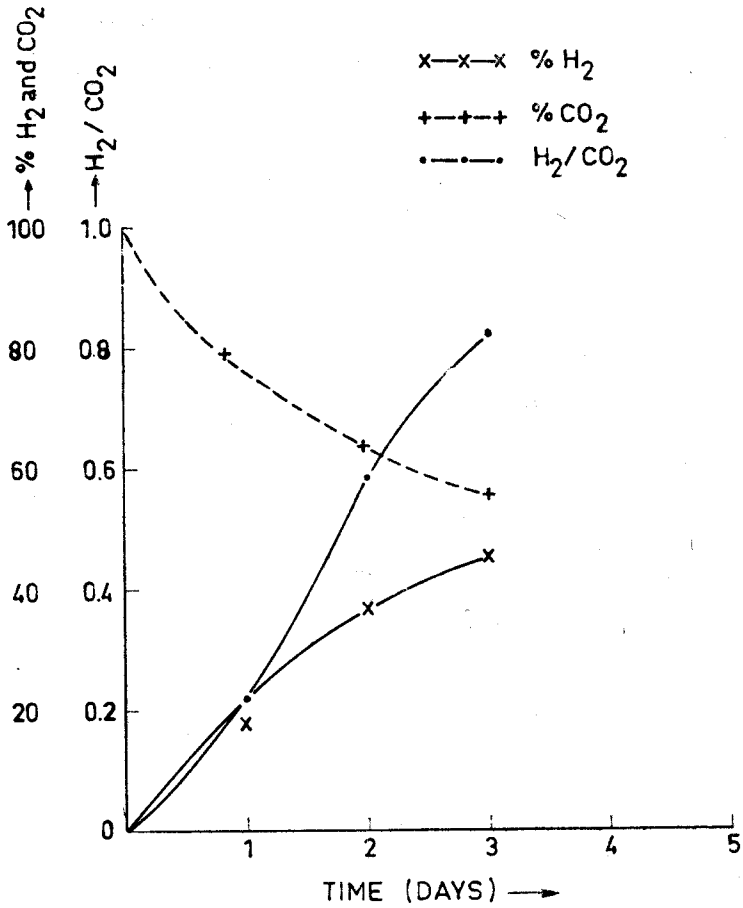


Figure - 6: The daily variations of the CO₂,H₂ percentage of the gas produced from pectin and the H₂/CH₂.

graphically in figures 3, 4, 5 and 6. These figures show the variations of the percentage of carbon dioxide, hydrogen and H_2/CO_2 ratio against time. They point out that the hydrogen content of the gas mixture increases continuously up to the 45 % and carbon dioxide decreases from nearly 75 % to 50 %. The H_2/CO_2 ratio increases from 0.33 in the first day of the fermentation to 0.96 in the last day. These results indicate the potentiality of the citrus albedo as an alternative source of hydrogen via anaerobic fermentation process. The production of different varieties of citrus is approximately 8×10^6 tons per year². Excluding the exported amount and considering, the albedo represents 50 % of the whole fruit, the yearly production of hydrogen will approximately be 4.2×10^6 cu m which means 2.52×10^9 TL/year in value by taking the present unit price of hydrogen as 600 TL/ cu m. This gas can either be used as fuel or as chemical in process industries.

If the molecular structure of the pectin is looked at closely, because of the available methoxyl groups, it can be suggested theoretically that gas mixture produced from the anaerobic fermentation of the pectin should contain methane gas. But the analyses performed did not show any trace of the methane gas. As the investigation of this phenomenon was out of the scope of this work it was not examined.

In conclusion it can be said that; the citrus residues which are considered as valueless waste material in fruit juice industry can provide an alternative source for the production of hydrogen gas amongst its other usage

ÖZET

Her türlü artık maddenin değerlendirilerek hem çevre sağlığının korunması ve hem de kullanılabilir ürünlerin elde edilmesi çağımızın önemli sorunlarından birisidir. Bu amaçla, çok değişik karaktere sahip artıklar ve çok değişik metodlardan yararlanılmaktadır. Bu artıklar arasında tarımsal olanların yeri büyüktür. Kullanılan metodlar arasında da fermentasyonun, bilhassa anaerobik fermentasyonun önemi fazladır. Çünkü bu yolla elde edilen ürünlerin enerji sağlayan gazlar olduğu bilinmektedir.

Bu çalışmada narenciye artıklarının ve pektinin anaerobik fermentasyonu incelenmiştir. Ulaşılan sonuçlara göre, narenciye artıklarının anaerobik fermentasyonu ile elde edilen gaz karışımında % 50 ye varan oranlarda hidrojen gazı bulunabilmektedir. Bu gaz istenirse yakıt olarak veya istenirse kimya endüstrisinin bir girdisi olarak kullanılabilir.

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