EXTRACTION AND FERMENTATION OF ENSILED SWEET SORGHUM

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WE HEREBY RECOMMEND THAT THE THESIS PREPARED UNDER OUR SUPERVISION BY KARL S. NOAH ENTITLED THE EXTRACTION AND FERMENTATION OF ENSILED SWEET SORGHUM BE ACCEPTED AS FULFILLING IN PART REQUIREMENTS FOR THE DEGREE OF MASTER OF SCIENCE.

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ABSTRACT

EXTRACTION AND FERMENTATION OF ENSILED SWEET SORGHUM

Sweet sorghum, variety Rio, was ensiled for 10 months prior to being subject to extraction studies using a pilot scale continuous countercurrent diffuser. An objective of the study was to see how control of liquid-solid ratio and diffusion temperature affected the recovery of sugars and organic acids from the ensiled sweet sorghum. Samples of the juice extracted from the sweet sorghum ensilage were fermented with Clostridium acetobutylicum and Saccharomyces uvarum to assure that no inhibitory or other detrimental substances were formed during the ensiling or extraction steps.

As the liquid-solid ratio decreased, the diffusion juice component concentrations increased, but never to a level of fermentable sugars suitable for fermentation work. For a given change in ensilage sugar concentration over the length of the diffuser, it was found that not all of the liberated sugar was removed in the diffusion juice. Thus, even though greater than 90 percent of the components of the sweet sorghum were extracted, less than 90 percent of these components ended up in the diffusion juice. Therefore, the diffuser operated at unsteady state. Diffusion temperature had no effect on extraction efficiency. A model was applied to help predict diffuser performance. Only selected cases were found to provide satisfactory predictions.

The organism best suited for fermentation of the concentrated ensiled sweet sorghum diffusion juice was C. acetobutylicum. The advantage of C. acetobutylicum was that in addition to sugars, it

utilized lactic acid, the major fermentation product of ensiling. However, the C. acetobutylicum fermentations did not exhibit the acid break point; thus, small amounts of butanol were formed. No conclusions were possible pertaining to the formation of inhibitory substances during ensiling or extraction.

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INTRODUCTION

The declining reserves and increasing costs of fossil fuels has lead to the investigation of biomass as as alternative source of liquid fuels and/or chemical feedstocks. Sweet sorghum is one of the most promising crops which can be grown for biomass in temperate climates. It is a genetically diversified, drought tolerant, adaptable, energy efficient plant, which produces high yields of fermentable sugars. Most research on sweet sorghum has been devoted to the potential of the sugar crop as a producer of ethanol.

Two serious drawbacks have prevented sweet sorghum from being widely utilized as an ethanol feedstock: storability and sugar extraction with currently available technology (10). An important factor in the use of renewable resources is the seasonal availability and storability of the raw material. Rapid deterioration of seasonal biomass allows its use for only a short portion of the year. Therefore, a reliable preservation method must be employed so that continuous use of the plant can be made. Whereas grain, the predominant ethanol feedstock, can be stored indefinitely, sweet sorghum vegetation tends to deteriorate after harvest (10, 35). Ensiling was used as a preservation method for sweet sorghum and allowed for storage periods of up to 155 days (32). It was determined that ensiling was a viable means of preservation and also enhanced the enzymatic hydrolysis of the combined hemicellulose and cellulose fractions.

Most proposed processing strategies for sugar extraction with state-of-the-art technology are based on separating and then fermenting the sugar fraction of the stalk. These processes are either uneconomical, energy inefficient or unproven on a commercial scale (20). The problem of extracting sugars from sweet sorghum in high yields while avoiding complex processing methods has not been solved (3). Over 90 percent of the sugar could be extracted using sugar mill equipment but these mills are extremely capital and labor intensive and would only be feasible for large alcohol producing systems (9). Using simple three-roll mills only around 50 percent of the sugars can be extracted (60).

Meade and Chen (42) in 1977 reported that modern sugar cane milling plants have been leaning toward the use of diffusers rather than the traditional roller mills for extracting the sugar from sugar cane because of the lower cost of diffusers and their relatively trouble-free operation. Countercurrent diffusers are also being used at present in the sugar beet industry. Some of the objectives of diffusion are to transfer the sugar from the stalk to the solution phase, as completely as possible, while keeping the maximum amount of nonsugars in the diffused pulp. These two objectives should be accomplished with the minimum dilution of the sugar. The rate of extraction in a liquid-solid system is affected by a number of independent varibles. A few of these are temperature, liquid-solid ratio, concentration of solvent, and particle size.

The overall objective of this study was to see how the control of two independent variables, liquid-solid ratio and temperature, affected the recovery of sugars and organic acids from chopped ensiled sweet

sorghum in a continuous countercurrent diffuser. Secondary objectives were twofold: 1) maximize recovery of fermentable fractions in a manner compatible with feasible fermentations, 2) ferment samples of extracted sweet sorghum ensilage with Clostridium acetobutylicum, Saccharomyces uvarum, and Zymomonas mobilis to determine product yields and to assure that no inhibitory or other detrimental substances would be formed during the ensiling or extraction steps.

LITERATURE SURVEY

1. Sweet Sorghum

1.1. Why use Sweet Sorghum

The selection of sweet sorghum as an alternative alcohol crop is based on several advantages: genetic diversity (24,41,43), climatic adaptation (3,24,29,40,42), and yield potential (3,18,24,41,51). Over 500 lines of sweet sorghum have been collected, representing a broad array of characteristics which may be useful for alcohol production (24). Sweet sorghum is normally a tropical crop, but hybrids have been developed with added cold tolerance and increased seedling vigor for production in temperate environments such as the midwest and northern states. Development of grain-sweet sorghum crosses have the potential for greater total fermentable carbohydrate yields by fermenting grain and sugars (24,43). These grain-sweet hybrids, also known as 'high energy' sorghums (43), combine the sugary stalk of the sweet sorghum with the large kernel production of the grain sorghum, which gives it the potential as a source of food, feed, fiber and/or fuel. With the diversity that exists in this species there could be even more improvement in the total fermentables contained within the stem (43). Also because of the diversity within the species, the nature of gene action and the ease of crossing, breeders can quickly attack some of the basic questions relating to potential utilization at all stages

along the development stream, from the agronomics to the actual alcohol fermentation (43).

Sweet sorghum can be grown from seed in almost all the states in the U.S. (43). Because of its relatively low water requirements and drought resistance, it has adapted to many otherwise submarginal areas (24,43). It is grown predominately in the mid-south and southeastern regions of the U.S., but it can be grown in almost any temperate climate which has about a 3 month growing season. However, yields vary widely with location and variety (5). Besides lower water requirements than grain crops (3,5) sweet sorghum also has lower soil fertility and nutrient requirements than grain crops (3,4,5,30).

Sweet sorghum produces high quantities of fermentable sugars and biomass which can be used for alcohol production (24,30,60). Compared with grain crops biomass yields for sweet sorghum are higher (3) and production yields of sugar exceed starch yields of corn and other grain crops (3,4). Sweet sorghum can produce slightly more ethanol than sugar beets and about 1.6 times more than corn as grain (30). Sweet sorghum has a potential ethanol yield of about 4000 liter/hectare (L/ha) (10,51). This compares with grain ethanol yields of 2290 L/ha for corn, 917 L/ha for wheat, and 823 L/ha for grain sorghum (51). Potential ethanol yields, according to Bryan et al. (5), for three high yielding varieties of sweet sorghum (Keller, MN 1500, and Ramada), and based on complete conversion of sugar to ethanol were 3960 to 4030 L/ha. Lamb et al. (30) gives a possible ethanol yield of 3560 L/ha from sweet sorghum. This yield was estimated at 7.6 tons/ha of fermentable sugars, which were assumed to be 16 percent of the fresh plant mass.

1.2. Components of Sweet Sorghum

Sweet sorghum is composed of cellulose (around 47 % (w/w)), hemicellulose, lignin, starch and sugars. The exact physical properties and chemical composition of sweet sorghum are dependent on a number of factors, such as variety, cultural practices, methods and time of harvest, and processing techniques.

The sugars can be converted directly to ethanol, whereas the cellulose, hemicellose and starches must first be broken down to sugars by chemical or enzymatic means. The sugars comprise 17 to 30 percent of the plant on a dry weight basis (28). The majority of the sugar is sucrose, approximately 70 to 75 percent of the total sugar (28), the rest being made up of glucose and fructose (28,42,14). Several constituents that are of secondary importance, but do contribute to the total biomass production of dry matter include protein, minerals, ash, fat and aconitic acid (28,43). These are of interest in evaluating the by-product potential of the stillage residues from ethanol fermentation as feed for animals. Aconitic acid may be important as a possible inhibitor of sweet sorghum juice fermentations (11,14).

2. Preservation of Sweet Sorghum

2.1. Why Preserve Sweet Sorghum

As mentioned earlier, one of the serious drawbacks of using sweet sorghum is its storability. Like other sugar crops, there is rapid deterioration during storage. This rapid deterioration of the sugars is suggested to be caused by the tissues' own respiration and autolysis and/or by microbial decomposition (18,48,49). The availability of

sweet sorghum is limited to no more than two or three months per year (i.e., the harvest season) because the crop cannot be stored without sugar deterioration. Therefore, a reliable preservation method is needed to make sweet sorghum available as a fuel crop all year long.

A study of post-harvest losses of fermentables from sweet sorghum was done by Eiland, Clayton and Bryan (17,18). One objective of their study was to determine fermentable sugar losses during a 1-week storage period at 25 to 30^{o}C for sweet sorghum, variety Wray, harvested by three methods. The three harvesting methods were: 1) hand cut whole stalks, 2) billeted sorghum (0.6 m billets) cut with a sugarcane harvester, and 3) chopped sorghum (0.5 to 5.0 cm lengths) cut with a forage harvester. Hand-cut stalks and billets did not deteriorate significantly during the one week storage, but chopped sweet sorghum lost 49 percent of its fermentable sugars.

Results of another study by Hansen and Ferraris (21) of the post-harvest losses of fermentables from sweet sorghum, variety Wray, support the above study. Whole stems were harvested and stored on the ground while they awaited milling. The total sugar content in the stems was 43 percent of the dry matter and did not significantly change during the post-harvest period up to 72 hours. However, sucrose decreased from 34 to 19 percent of the dry matter within the first 48 hours, while monosaccharides increased correspondingly from 9 to 23 percent of the dry matter. This suggests that sorghum stems should be preserved for sugar production within the first 24 hours for maximum sucrose content. Inversion to reducing sugars (monosaccharides) after 24 hours may render the crop more suitable for use as an industrial feedstock (e.g. liquid fuel production).

2.2. Preservation Methods

2.2.1. Aerobic Storage Under Cold Ambient Temperatures

A continuing study by Parrish et al. (48,49) investigated the post-harvest preservation/loss of fermentables from sweet sorghum when stored under ambient conditions during cool Appalachian falls and winters. In the first part of the study, October 1982 to January 1983, sweet sorghum, variety Dale, was stripped, cut, and bound into shocks of 0.5 or 1.0 m diameter (packing density of both sizes of bundles was 400 kg/m^3). Bound bundles of each size were stored in an open shed or fully exposed to the elements. After 3 months of storage, there was no significant decline in moisture content of the stems inside the bundles, but fermentables declined significantly, from 48 percent to 35 percent of the dry matter. Location of bundles had no effect on the preservation of fermentables. Stems in the larger bundles retained more of their fermentables than stems from the smaller bundles (38% vs. 33% of the dry matter after 3 months).

For the second part of the study, they looked at more varieties, Dale, M81E, Keller, and Wray, bound in 0.5 or 1.0 m diameter bales for 150 days (November 1983 to April 1984). Within the small bales, mean moisture content dropped from 75 percent at harvest to 65 percent after 150 days. Average fermentables fell from 53 to 38 percent of the dry matter, but there was no significant decline until 120 days of storage. Within the larger bales, mean moisture content dropped from 77 percent at harvest to 70 percent after 150 days, but did not decline significantly until after 120 days. Average fermentables fell from 50 to 34 percent of the dry matter, but with no significant decline until 150 days.

2.2.2. Sulfur Dioxide Treatment

Since the sulfite group is used in many food processing activities as a preservative, Eiland et al. (19) studied the effectiveness of sodium metabisulfite and sulfur dioxide as preservatives to reduce fermentable sugar losses in stored sweet sorghum at ambient storage temperatures. Experiments were performed with either variety Wray or Rio as stalks, billets, or chopped sorghum. Levels of sodium metabisulfite (powder) or sulfur dioxide (gas) necessary to produce sulfur dioxide concetrations of 500, 1000, 2000, 3000, and 4000 ppm (0.05, 0.1, 0.2, 0.3, and 0.4 % wet basis) were used. They found that sulfur dioxide gas was a better preservative. Long term storage of stalks and billets with sulfur dioxide levels above 3000 ppm did not appera successful. Sulfur dioxide levels above 3000 ppm preserved chopped sweet sorghum and adequately retained the fermentable sugars up to 4 months. Addition of 5000 ppm of sulfur dioxide extended the storage time to 6 months. Lower concentration levels only provided protection for a short period of time. Fermentation of juice from the preserved sweet sorghum was successful when the sulfur dioxide was neutralized with lime.

A subsequent study by Eckhoff et al. (15) investigated at five storage temperatures, -16, 2, 12, 22, and 32^{o}C, higher sulfur dioxide (gas) levels (0.5, 1.5, and 3.0 % wet basis) on the preservation of chopped sweet sorghum of the Rio variety. They found that dosage levels of 0.5 percent sulfur dioxide and above maintained sugar levels during 3 months of storage over the temperature range studied. Losses of over 75 percent of the soluble sugars were observed in untreated stored samples. Another observation from their study was that at

-16°C, sulfur dioxide dosages of 1.5 and 3.0 percent inhibited enzymatic hydrolysis of sucrose to glucose and fructose.

Although use of sulfur dioxide was successful at increasing storage time, a disadvantage is its toxicity. Sulfur dioxide is an irritant to mucus membranes and forms sulfuric acid in the lungs. Therefore, sulfur dioxide must be treated as a hazardous material.

2.2.3. Ensiling

Ensiling is a commonly practiced agricultural method for the preservation of chopped forage materials. During ensiling, the organic acids produced from soluble sugars by the Lactobacillus and Streptococcus bacteria may cause hemicellulose-lignin sheathing to break down. As a result the accessibility of water to cellulose for hydration and of enzymes for hydrolysis is reportedly improved (35). Therefore, Linden et al. (32,33,35,37) investigated ensiling as a storage and pre-processing technique for sweet sorghum.

Earlier work (31,34) showed ensiling to be a low energy process whereby the biomass can be preserved and the lignocellulosic fraction may be rendered reactive to hydrolysis with cellulases and hemicellulases to yield additional fermentable carbohydrates. In the initial study by Linden, Moreira, and Smith (35), results indicated that when fresh sorghum was ensiled, the resulting disruption of the cellulosic fractions allowed 70 percent hydrolysis to fermentable sugars. This was contrasted to the low conversion of the cellulose when enzymatic or acid hydrolysis was attempted on the raw material. Thus, ensiling permitted a more complete hydrolysis and a considerable savings of energy over more commonly used pretreatment/hydrolysis systems that require extensive heating before and during hydrolysis.

Such systems include the alkaline-oxygen treatment, autohydrolysis and organosolvent pulping.

In a subsequent study by Linden et al. (32), two different procedures were examined prior to ensiling. The first procedure was wilting the sweet sorghum to a suitable moisture content of 66 percent and the second procedure was pressing the sweet sorghum on a manual three roll apparatus to express cell sap and reduce the moisture content to 66 percent. Both the pressed and wilted materials were then examined for ensiling periods up to 155 days with the addition of 0.5 g ensiling inoculum per kg of wet sorghum (Pioneer Hybrid Silabac). When the sweet sorghum was ensiled, either with or without prior pressing, the sucrose was rapidly converted to invert sugars and lactic acid; approximately 65 percent of the invert sugars were conserved indefinitely. In addition, the acidic environment produced by the ensiling served as a pretreatment for cellulose conversion. Compared to non-ensiled material, a 44 percent increase in the reducing sugar yield from enzymatic hydrolysis (four International Units cellulase activity per gram of dry material) of constituent cellulose was observed in samples of pressed sorghum which had been ensiled for at least 15 days.

In their next study, Linden et al. (33,34) looked at in situ cellulose hydrolysis during storage of sweet sorghum by ensiling. Ensiling was conducted with Pioneer Hybrid Silabac 1177 ensiling inoculum (at 1 percent on a dry weight basis). Simultaneously, enzymatic hydrolysis was conducted with cellulose and hemicellulose degrading enzyme preparations at concentrations of 0.0, 0.01, 0.1, 1, and 10 International Units of cellulase activity per gram of dry

material, which were added at the time of inoculation. The extractablereducing sugars produced as a function of ensiling time can be seen in Figure 1. The results show a proportionality between an increase in fermentable sugar extraction and the dosage of cellulase supplied. Therefore, *in situ* conversion of cellulose during ensiling did occur.

Linden *et al*. have shown that ensiling is a low energy process whereby the biomass can be preserved (65 % of original sugar for over one year) and the lignocellulose fraction rendered reactive to hydrolysis with cellulase and hemicellulases to yield additional fermentable carbohydrates. Thus, low-technology, long-term storage, pretreatment and conversion of available carbohydrates in sweet sorghum can be conducted in one conventional farming operation ensiling.

2.3 Effects of Ensiling on Sweet Sorghum

As seen earlier, sweet sorghum contains cellulose, hemicellulose, lignin, starch, sucrose , glucose, and fructose. Cellulose is a homogeneous polymer of glucose, which when hydrolyzed by acidic or enzymatic catalysis, yields glucose. Hemicellulose molecules are often polymers of pentoses (xylose and arabinose), hexoses (mannose and galactose) and a number of sugar acids. Hemicellulose is more easily hydrolyzed than cellulose under mildly acidic conditions. Lignin is a polyphenolic macromolecule which acts as a binder for the cellulose and hemicellulose (polymers) and is not easily depolymerized.

The two objectives of ensiling are maintaining anaerobic conditions and preventing the growth of clostridia. Anaerobic conditions are maintained by storing chopped material in an air-tight container or by

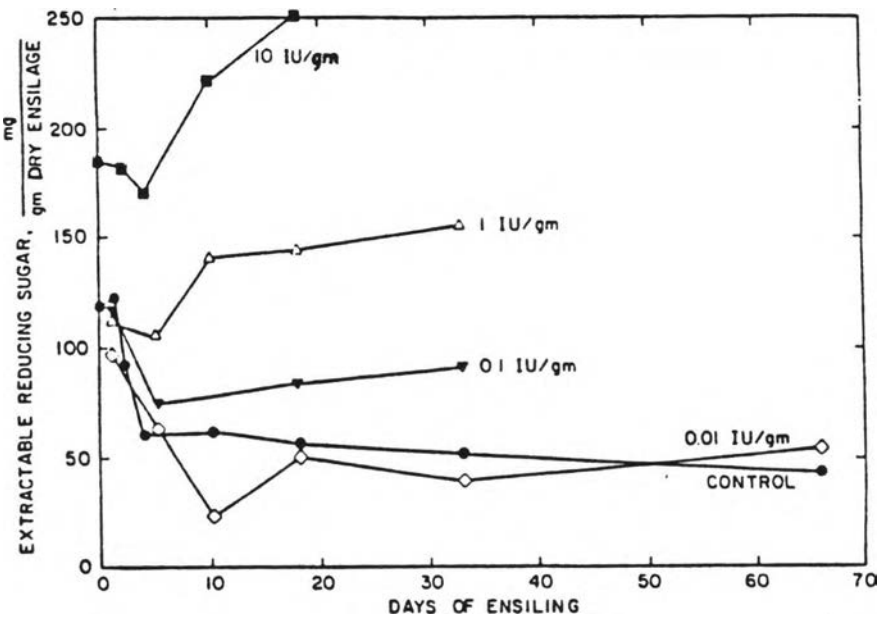


Figure 1. Reducing sugars extracted from sweet sorghum ensilage versus ensiling period as a function of various levels of cellulase enzyme complex added at the time of inoculation (37).

compaction. If oxygen is allowed to enter the silo, rapid deterioration occurs because of aerobic microbial activity, and the material becomes inedible and frequently toxic. The inhibition of clostridia is important because these organisms produce butyric acid and degrade amino acids to undesirable products. Clostridia thrive in anaerobic environments, but are inhibited by low pH, high temperature, and low moisture content. The most common method of preventing the growth of clostridia is by the promotion of lactic acid fermentation by wilting or the addition of lactic acid producing bacteria. The lactic acid bacteria are added to the silos where it readily utilizes the plant's sugar to produce lactic acid, aerobically or anaerobically. The production of lactic acid inibits the growth of clostridia by lowering the pH and preserves nutritionally important amino acids.

During ensiling sugars may be released from polysaccharide hydrolysis (39). Not only do plant enzymes liberate pentose sugars, mainly xylose and arabinose, from hemicellulose, but acid hydrolysis also plays a part. Acid hydrolysis of hemicellulose also produces acetic acid by deesterification of acetyl-substituted sugars (16). Hydrolyzed hemicellulose will also produce galactose, a component of hemicellulose. Oligosaccharides can possibly come from acid hydrolysis, caused by ensiling conditions, of polysaccharides such as cellulose, hemicellulose, and starch. In the paper by Eckhoff et al. (15), HPLC chromatograms showed early broad peaks which represented macromolecules such as starch, cellulose, and soluble salts which were not quantified.

Lactobacillus and Streptococcus are two lactic acid producing bacteria commonly associated with ensilage (39, p.62). Sucrose, the

primary sugar component of sweet sorghum, is acid hydrolyzed to the invert sugars, fructose and glucose, by the acid conditions resulting from the metabolism of the lactic acid bacteria (36). Due to the Lactobacillus or Streptococcus metabolism, glucose is converted to acetic acid, lactic acid and ethanol. Fructose is converted to mannitol, lactic acid, and ethanol (fig. 2; 39, p.70). Small amounts of propionic and butyric acids are frequently found in lactate silages, the concentration of butyrate depending very much on the rate at which lactate is produced (39, p.181).

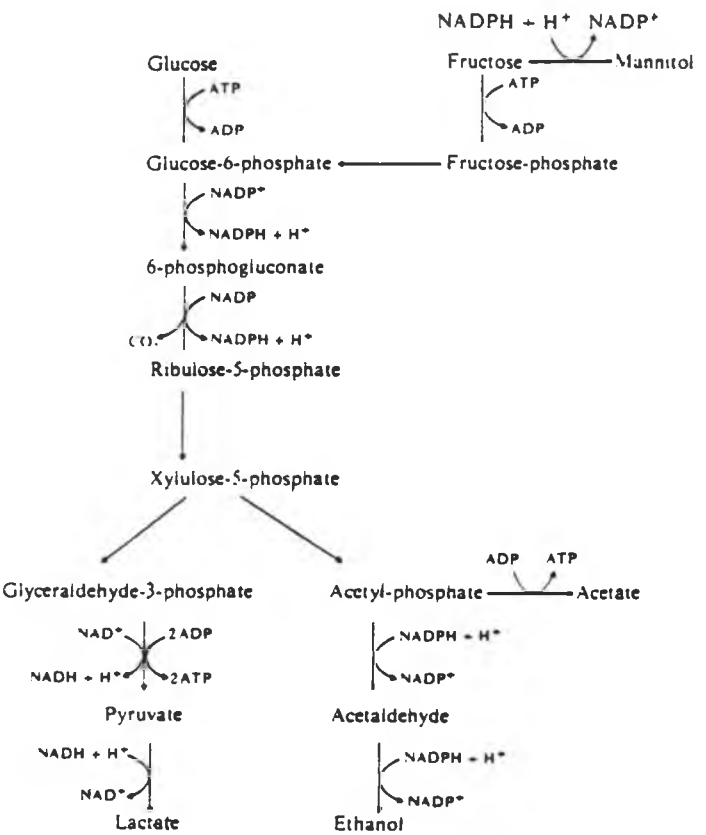
3. EXTRACTION PROCESSES FOR SWEET SORGHUM

Two common methods of sugar removal are mechanical expression (by pressing) and diffusional extraction either batchwise or in a continuous fashion.

3.1 Expression

Mechanical expression is usually performed with 3-roll mills. This can be done with small processors consisting of one 3-roll mill or on a large scale, such as practiced in the sugar cane industry, where multiple pressings are performed with 5 or more 3-roll mills.

The small scale 3-roll mills are generally inefficient and have extraction rates below 50 percent of the sugar contained in the stalks (5,51,60). Juice yeilds for small 3-roll mills of 55 to 57 percent of sweet sorghum stalk weight were reported by Lamb et al. (30) and Reidenbach and Coble (51). In extraction tests of farm scale mills, using eight varieties of sweet sorghum, juice yield did not exceed 40



Sum: (1) $C_6H_{12}O_6$ (glucose) + ADP → $CH_3CHOHCOOH + C_2H_5OH + CO_2$ + ATP

(2) $3C_6H_{12}O_6$ (fructose) + H_2O + 2ADP → $CH_3CHOHCOOH + 2C_6H_{14}O_6$ + $CH_3COOH + CO_2$ + 2ATP

(3) $C_6H_{12}O_6$ (glucose) + $2C_6H_{12}O_6$ (fructose) + H_2O + 2ADP → $CH_3CHOHCOOH$ + $CH_3COOH + 2C_6H_{14}O_6 + CO_2$ + 2ATP

Figure 2. Fermentation of glucose and fructose by heterofermentative lactic acid bacteria (39).

percent of stalk weight and contained less than half the total sugars originally in the stalk (5). Even with an improved mill which had intermeshed grooved rolls, the highest juice yield did not exceed 60 percent, and the new rolls were 16 percent more efficient than the orignal rolls (45). Monroe et al. (46) reported a maximum juice extraction of 47 percent from sweet sorghum stalks using a small horizontal roll mill.

A traditional sugar cane extraction facility using roller mills, uses at least 5 or more rolls in series with warm water being sprayed in countercurrent fashion on the bagasse (exhausted cane), to insure complete removal of sugar. This process is called compound imbibition. These large mills are very efficient recovering over 95 percent of the sugar from the cane. However, they are extremely capital intensive (51) and consume considerable power (23).

3.2 Diffusional Extraction

Meade and Chen (42) reported that modern sugar cane milling plants have started to use diffusers rather than the traditional roller mills for extraction of sugar because of the lower cost of diffusers and their trouble-free operation. Another advantage of diffusers is high extraction efficiency (60). Cotton et al. (11) reported over 98 percent of the sucrose present in sorghum could be extracted by diffusion, utilizing a 14 cell diffusion battery, previously employed in the sugar beet industry (40,56), but only 85 percent could be recovered with four passes through a small cane press with the use of 35 percent imbibtion water.

3.3 Alternate Extraction Processes for Sweet Sorghum

The need for improved small scale processing methods over the commercially available technology of roller mills has led to several new approaches for sugar extraction. These would include using a Tilby separator to remove stalk pith and fermenting juice pressed from the pith, and using the EX-FERM process in which sugars from small chips are extracted and fermented simultaneously in an aqueous solution.

In the Tilby system billets of the stalks are split lengthwise and the inner pith is scraped and separated from the outer rind fiber to form two usable products (50). Juice containing fermentable sugar is recovered from the pith by alternate squeezing and water leaching, while the rind can be used to manufacture fibrous products. The idea behind the Tilby process is that the pith contains 85 percent or more of the sugars present in the stalk (53). This process has not been commercialized because no practical method has been found to singulate stalks from bulked material (12). Another limitation of the system is the necessity of supplying stalks with low trash levels (51). Reidenbach and Coble (51) found that extracting the pith with a Tilby processor was not a practical processing method. Neither harvesting machinery compatible with the Tilby processor nor a large scale processor has been successfully demonstrated. Also the pith was very fibrous and could not be pumped easily, nor did it ferment well.

In the EX-FERM process (8,9,54,55) dried stalks are chopped into small chips about 0.5 to 1.0 cm in size. Water is added to the chips in sufficient quantity to have all solids submerged and a yeast inoculum added. The fermentation proceeds as the sugars diffuse from the tissue cells into the bulk solution. When the fermentation is

complete, the liquids (ethanol-yeast aqueous suspension and juice from pressed chips) are separated from the solids and used to extract and ferment a new batch of fresh or previously dried chips. In this manner, sucrose extraction and fermentation are cyclic, and consecutive steps are carried out in one operation, thus the name EX-FERM. Repetition of this cycle could continue until either the final concentration tolerated by the yeast strain used is reached or ethanol yields are reduced below acceptable levels due to microbial contamination. Both the solids and liquids are distilled to recover ethanol. Fermentations utilizing 98 percent of the sugar are typical.

3.4. Solid-Substrate Fermentation of Sweet Sorghum

Fermentation processes for sugar crops have recently been proposed that avoid traditional pressing and countercurrent leaching steps used to separate juice prior to fermentation. Such processes may be especially attractive for small-scale ethanol production by farmers or cooperatives because of lower capital investment. One of these fermentation processes would be the EX-FERM process aforementioned and the other would be solid-substrate fermentation (SSF). Of the two methods, the EX-FERM process requires larger reactor volumes and is more energy intensive. Moreover, the EX-FERM process requires a higher level of technology and is not as suitable for use on the farm site (25).

The advantages of using SSF rather than submerged fermentation of sweet sorghum are (20,26): 1) greater fermentor productivity (ethanol production per unit volume); 2) reduced reactor volume, which results in lower capital and operating costs and lower space requirements; 3) reduced need for nutrient addition; 4) lower volumes of stillage for

disposal; 5) less energy for distillation; 6) lower chance of contamination of fermentation medium since moisture content is lower than in conventional submerged fermentations; and 7) easier product recovery.

The objective of a study by Bryan and Parrish (6) was to carry out SSF of chopped sweet sorghum (variety Wray) and sweet sorghum juice and compare their rates of fermentation and yields of ethanol produced. SSF were conducted for 63 hours in 7-liter styrofoam pail fermentors lined with polyethylene bags. The fermentors contained about 3.5 kg of inoculated chopped sweet sorghum of either 0.6 or 2.5 cm chopped length. Inoculation was carried out in a cement mixer by addition of 0.3 percent DADY yeast as a water slurry (7 g water per g dry yeast). The fermentors were stored at ambient temperature and weighed periodically to estimate the rate of fermentation from weight loss. Juice for fermentations was expressed in a laboratory cage press from chopped sorghum. Fermentations of 250 ml juice were conducted in 500 ml erlenmeyer flasks inoculated with 0.1 percent DADY yeast. The flasks were placed in a 33^{o}C shaking incubator and weighed periodically to estimate fermentation rate. Theoretical yields of ethanol during fermentation were calculated assuming that the weight loss was caused entirely by stoichiometric conversion of sugars to ethanol and carbon dioxide (0.4885 g CO_2 lost per g sugar fermented).

They found that rates of fermentation and ethanol yields were higher for SSF than for juice fermentations. Rates and yields were similiar for either the 0.6 or 2.5 cm chopped sorghum SSF. The rate of SSF was highest initially with an average initial slope of the yield curves of 5.6 and 5.1 percent conversion of sugars to ethanol per hour

for the 0.6 and 2.5 cm chopped sorghum, respectively. After 63 hours, both chopped sorghum SSFs had 80 percent ethanol yields. For the juice fermentations, average initial slope of the yield curves was 3.8 percent conversion of sugars to ethanol per hour and had an ethanol yield of 73 percent after 58 hours.

A study by Kargi et al. (26) investigated SSF of chopped sweet sorghum for ethanol production in static flasks using Saccharomyces cerevisiae (NRRL Y-11572). The influences of various process variables such as moisture content, temperature, yeast cell concentration and nutrient medium composition on the rate and extent of ethanol fermentation were investigated.

Initial experiments were performed to compare SSF of sweet sorghum particles with conventional fermentation of the sorghum juice obtained from an equivalent amount of sorghum. The juice was removed by squeezing the sorghum. Approximately 25 ml of juice was obtained from 50 g of sorghum. Three fermentations were performed, one containing both the presqueezed solids and juice, one containing unsqueezed sorghum, and one containing only 25 ml of juice. After sterilization, 25 ml nutrient medium and 5 ml inoculum culture were added to each flask, and the flasks incubated at 28^{0}C and 200 rpm. The final ethanol concentration achieved by fermentation of juice was only 2.2 percent, lower than that obtained for both the presqueezed solids and juice and unsqueezed sorghum, which both had final ethanol concentrations of 3.5 percent. Thus, SSF of sweet sorghum may provide a means of utilizing a greater percentage of the total sugars in the sorghum plant without further dilution of the sugars. Extractive techniques for removing the sugars in the plant would result in

dilution of the sugars and the product and causes higher product recovery costs.

One important factor that affects the performance of SSF is the moisture content of the solids. Reduction of moisture content of chopped sorghum before fermentation is advantageous since the chance of contamination of fermentation medium is reduced by reducing moisture content of solids, and the final ethanol concentration in the medium is higher at low moisture levels. However, there is a lower limit of moisture content below which yeast cells may not function to produce ethanol. As a general trend, Kargi et al. (26) found that a reduction in initial moisture content of sorghum resulted in a higher final ethanol concentration in the liquid medium (juice pressed from solids). The optimal moisture content of 70 percent resulted in a 6.7 percent final ethanol concentration in the juice and a rate of ethanol fermentation of 6 g ethanol per liter of liquid medium per hour.

A set of shake flask experiments was performed in order to determine if nutrient salts addition was necessary to ferment sorghum juice. Addition of mineral salts to the sorghum juice did not improve the fermentation yield of the juice by the yeast cells. The sorghum juice appeared to contain the necessary nutrients for growth of yeast cells.

Finally, a set of static flask experiments were performed to investigate the influence of temperature and initial cell concentration on the rate and yield of ethanol fermentation from SSF of sweet sorghum at 70 percent moisture. The rate of ethanol fermentation increased with increasing temperatures in the range of 20 to 35^{o}C and remained unchanged in the range of 35 to 45^{o}C. The optimal temperature

resulting in the highest rate of ethanol formation was 35°C. The influence of yeast cell concentration experiments were performed at 35°C. An increase in the initial cell concentration from 10^7 to 7 X 10^8 cells/g sorghum resulted in an 1.8 increase in the rate of ethanol formation from 5.8 to 10.4 g ethanol per liter juice pressed from solids per hour. However, the same increase in the cell concentration resulted in only a 5 percent decrease in final ethanol concentration, a decrease from 7.6 to 7.2 percent ethanol.

In a subsequent study by Kargi and Curme (25), SSF of sweet sorghum to ethanol in a rotary-drum fermentor was investigated. A rotary drum offers a simple, inexpensive means for mixing the fermenting sorghum, thus ensuring homogeneity and effective heat transfer. The purpose of this investigation was to determine the influence of continuous mixing on fermentation rate, ethanol yield, and maximum ethanol concentration for SSF of sweet sorghum.

The SSF was conducted with 500 g of freshly chopped sorghum (0.2 to 7.0 mm) dehydrated to a moisture content of 65 percent by weight after addition of yeast inoculum (25 ml of inoculum at a cell concentration of 4 X 10^9 cells/ml). The rotary-drum fermentor was operated for 70 to 75 hours at 35°C at various rotational rates (rpm) of the drum.

The maximum rate and extent of ethanol formation obtained in the rotary-drum fermentor were 3.1 g ethanol/L/hr (based on expressed juice) and 9.6 g ethanol/100 g mash, respectively, at 1 rpm rotational speed. The rate of ethanol formation decreased with increasing speed of the rotary-drum fermentor. Ethanol yields obtained in SSF of sweet sorghum were about 80 percent of the theoretical yield.

A study by Gibbons et al. (20) investigated the use of a semicontinuous SSF device for production of ethanol from sweet sorghum. It consisted of a nonported steam pasteurization chamber to destroy bacterial contaminants in the shredded sweet sorghum, a yeast inoculation port, and an auger that simultaneously conveyed and mixed the fermenting pulp.

In the process, dried and shredded sweet sorghum (2.54 cm) was rehydrated to 70 percent moisture, acidified to pH 2.0 or 3.0, and either pasteurized (12 hours at 70 to 80^{o}C) or not pasteurized before spray inoculation with a broth culture of Saccharomyces cerevisiae (0.1 ml/g of wet pulp at 10^8 cells/ml). The auger was large enough to accomodate seven batches of acidified and inoculated pulp (1.66 kg per batch) at any one time. With or without pasteurization, the procedure of acidifing and inoculating a batch of pulp was repeated at 12 hour intervals for up to 400 hours. Due to the length of the auger and its slow rate of rotation, entering pulp did not exit from the fermentor for 72 hours.

Two operational modes were investigated; the first in which dried and ground sweet sorghum pulp was rehydrated, acidified and pasteurized before yeast inoculation, and the second in which sorghum pulp was rehydrated and acidified but was not pasteurized before inoculation. In each operational mode, 44.8 kg of pulp was processed through the fermentor during a 400 hour period. This amounted to 27 separate 1.66 kg batches, each with a retention time of 72 hours. For both operational modes, the ethanol concentration in the fermented pulp exiting the fermentor was approximately 6 percent (v/v), which corresponded to an ethanol yield of 179 liters/10^3 kg of dry sweet

sorghum, or 85 percent of the theoretical yield. Therefore, pasteurization had no effect on ethanol yields.

3.5. Why Diffusional Extraction

In review, 3-roll mills are inefficient only removing approximately 50 percent of the sugars from sweet sorghum. Larger scale roll mills such as that used in the sugar cane industry are efficient in sugar removal but are extremely capital intensive. The Tilby system was found to be an impractical processing method due to uncompatible harvesting machinery. Diffusers, on the other hand have high sugar extraction efficiencies, are of lower cost than the traditional sugar cane roller mills, and have relatively trouble-free operation.

4. COUNTERCURRENT EXTRACTION

In theory the most efficient extraction is obtained by using the countercurrent principle. The countercurrent multistage system allows high recovery of solute with a highly concentrated product solution because the concentrated solution leaves the system after contact with fresh solids (50). Multistage countercurrent contact between the solids and solvent may be obtained by actual movement of the solids by some means, countercurrent to the direction of solvent flow from stage to stage. Such extraction may be simulated with a number of stages in which solids remain stationary but are subjected to a multiple number of contacts with extracts of diminishing concentrations. A way to move the solids would be with a screw-type diffuser, such as the Silver D.d.s. slope diffuser or the Bruniche-Olsen diffuser used extensively

in the sugar-beet industry (40,56). An example of the batch countercurrent system would be the extraction or diffusion batteries formerly used in the sugar-beet industry (40,56). The diffusion batteries have been replaced by continuous diffusers due to complicated installation and operation of the batteries and efficiency and saving of manpower of the continuous diffusers (23).

The objectives of diffusion are to completely as possible transfer the sugar from the fresh plant material to the solution phase or diffusion juice, and leave the maximum amount of impurities in the diffused pulp (exhausted plant material). Some variables that can be controlled to affect sugar loss in the diffused pulp (an indirectly controlled variable), are: extraction temperature, retention time, solid dimensions, and liquid-solid ratio (L/S ratio).

The mathematical theory of diffusion in isotopic substances is based on the hypothesis that the rate of transfer of diffusing substance through unit area of a section is proportional to the concentration gradient measured normal to the section. Hence the first law of diffusion is:

$$F = - D \frac{\partial C}{\partial X} \qquad (1)$$

where F is the rate of transfer per unit area of section or flux, C is the concentration of diffusing substance, X is the space coordinate measured normal to the section, and D is the diffusion coefficient. The negative sign arises because diffusion occurs in the direction opposite to that of increasing concentration.

Liquid and solid diffusivities are strongly concentration dependent and generally increase with temperature (1a). The most common basis

for estimating diffusion coefficients in liquids is the Stokes-Einstein equation:

$$D = \frac{K\ T}{6\pi\mu R} \qquad (2)$$

where K is Boltzmann's constant, T is the absolute temperature, u is the solvent viscosity, and R is the solute radius. Equation 2 is derived by assuming a rigid solid sphere diffusing in a continuum of solvent, an infinite dilute solution (1 p.514,13). Even though the equation was derived under certain assumptions, use of this relationship will help explain the effects of temperature and particle size on the rate of diffusion.

As seen from equation 2, as the temperature increases, the diffusion coefficient is increased and thus the rate of transfer of the diffusing substance is increased. The effect of temperature on diffusion is explained by kinetic theory of solutions; with increased temperature, the velocity of molecules rises and viscosity of the solvent diminishes. The result is that during diffusion, molecules of solute are able to move easier between the molecules of the solvent (56,p.127).

An increase in temperature results in greater rates of diffusion of sugars from sweet sorghum, but also further extraction of soluble impurities. High temperature is essential to minimize bacterial action in the diffuser, but a temperature in excess of about $70^{o}C$ will cause solubilization of starch from chipped sorghum which would seriously interfere with recovery of sucrose (11,15).

As the Stokes-Einstein equation suggests the solid dimensions affect the rate of diffusion; the smaller the particle, the larger the

diffusion coefficient. Since time is required for sugar to diffuse through a given distance, advantage is gained by shortening the distance. By making the sweet sorghum particle smaller, there is more surface area available for extraction per unit weight of material. Thus, smaller particles increase the diffusion rate and allow for more complete extraction.

Diffusion is a slow process, the diffusivity of sucrose in the sweet sorghum tissue is 10E-6 square cm./s at 50^{o}C (60). For particles 2 mm thick, a 90 percent reduction of sucrose from the particle will require almost 2 hours of extraction. Thus, the longer the diffusion time, the more complete the extraction.

The liquid-solid ratio (L/S ratio) is the mass of fresh solvent (diffusion water) over the mass of solids. Larger L/S ratios will allow the average concentration gradient between the solid material (sweet sorghum) and the solvent (water) to remain high, thus allowing more complete extraction. Remember the diffusion process is controlled by the gradient of concentration as shown in equation 1. Even though use of large L/S ratios allows more complete extraction, there is a dilution effect on the concentration of the substance of interest. After the diffusion process, unit operations such as evaporation may be necessary to get the desired concentration. There is a trade-off involved with L/S ratio; larger L/S ratios allow for more complete extraction but have a dilution effect on the substance of interest. Therefore, a L/S ratio should be found at which most of the substance of interest is removed and at a suitable concentration for desired use, to avoid costly downstream operations such as evaporation.

5. POSSIBLE FERMENTATIONS UTILIZING SWEET SORGHUM JUICE

Three possible fermentations utilizing sweet sorghum juice to produce chemical feedstocks and/or liquid fuels are Clostridium acetobutylicum, Saccharomyces uvarum, and Zymomonas mobilis.

5.1. Clostridium acetobutylicum

The major products of the anaerobic Clostridium acetobutylicum fermentation are acetone, butanol, and ethanol with simultaneous production of H_2 and CO_2 gases as seen in Figure 3. Butanol is formed from butyric acid, while acetate is the precursor of ethanol and acetone. The normal solvent ratio is approximately 6:3:1, butanol, acetone, ethanol respectively. However, the relative proportions of each of these products in the fermentation depends on the fermentation conditions (e.g. pH, substrates, medium nutrients, agitation rate, temperature).

Duration of a conventional batch fermentation varies from 36 to 72 hours and occurs in two phases. From the typical growth and production formation curves (fig. 4), it can be seen that the whole batch process consists of 2 phases corresponding to the 2 stage product formation mechanism involved. The initial phase is characterized by cell growth and conversion of sugars into acids (e.g. acetic and butyric acid). Optimal pH for growth is 6.5, whereas solvent production is favored at pH 4.5 to 5.0. Preferred temperature for both growth and solvent production is 37^0C. During the initial phase the pH decreases due to the formation of acetic acid and butyric acid. During the final phase, the pH value increases, unless controlled, through the metabolism of

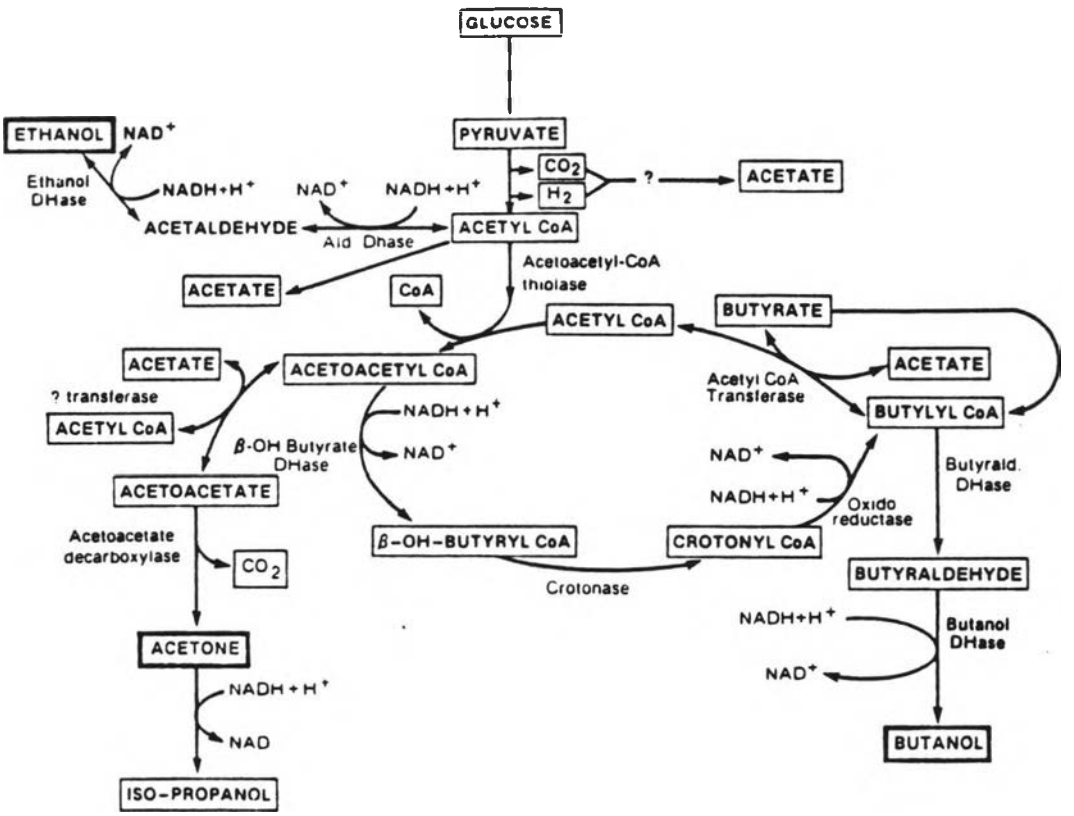


Figure 3. Main biochemical pathway leading to accumulation of acetone, butanol, and ethanol in the fermentation broth (63).

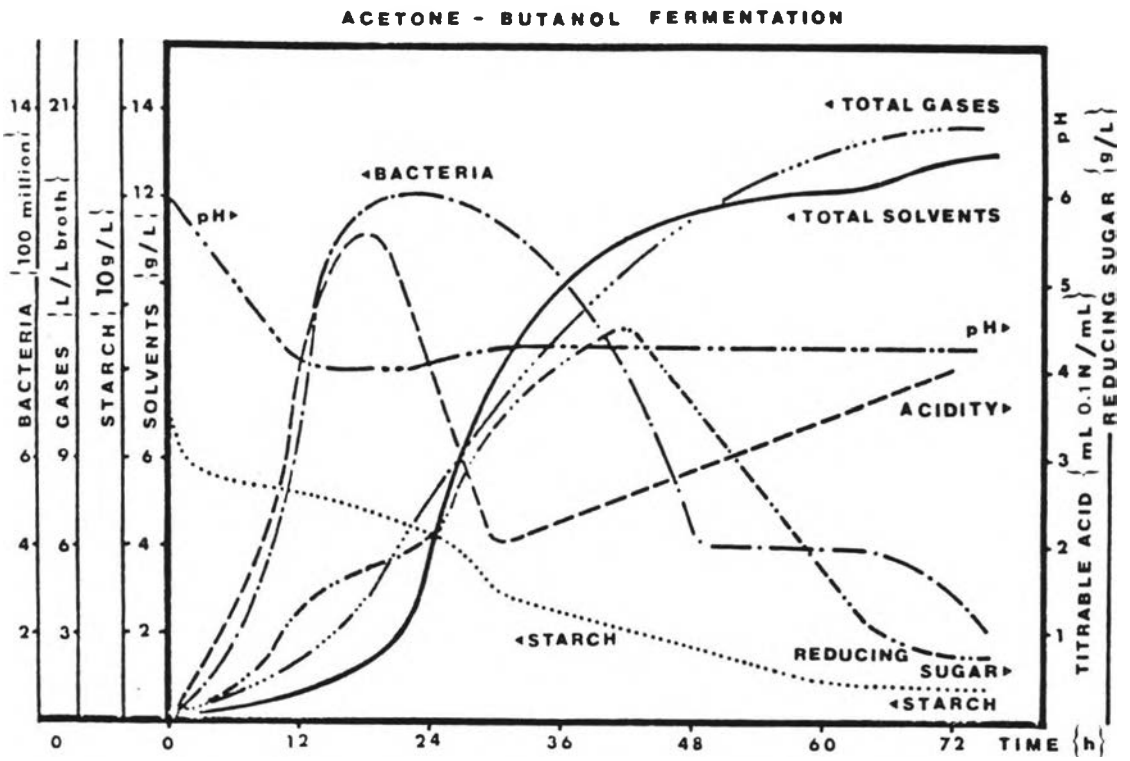


Figure 4. A typical profile of process parameters in the batch acetone-butanol-ethanol fermentation as carried out by Clostridium acetobutylicum (63).

these acids to ethanol, acetone and butanol. This fermentation is also product inhibited at 2 to 3 weight percent total solvents (butanol, acetone, and ethanol).

Some of the preferred sugars for butanol production are glucose, sucrose, cellobiose, fructose, maltose, and mannose (61). C. acetobutylicum is also capable of utilizing arabinose and xylose (47,63,64). Studies by Linden et al. (34) have shown that C. acetobutylicum is capable of using lactic acid in sweet sorghum ensilage extracts as a substrate.

5.2. Saccharomyces uvarum

The major products of the Saccharomyces uvarum fermentation are ethanol and CO_2 (fig. 5). It is important to avoid aerobic metabolism which utilizes glucose but does not produce ethanol. During initial stages of the fermentation oxygen ordinarily should not be totally eliminated as it is required for cell growth.

Sugars metabolized by Saccharomyces can be seen in Table 1. The theoretical yield of ethanol is 0.51 g ethanol per g glucose and 0.538 g ethanol per g sucrose. For S. uvarum, the optimum growth temperature is 25°C and the maximum temperature is 33.5°C (58).

The effect of pH on ethanol production varies with the type of system being used. Generally, pH values between 3.5 and 6.0 are employed with an optimum of 4.5 being reported for free yeast cell suspensions (65).

Ethanol is inhibitory to yeast at high concentrations, but ethanol tolerance differs depending on the strain. For most yeasts, the effect of ethanol inhibition is negligible at low alcohol concentrations (less

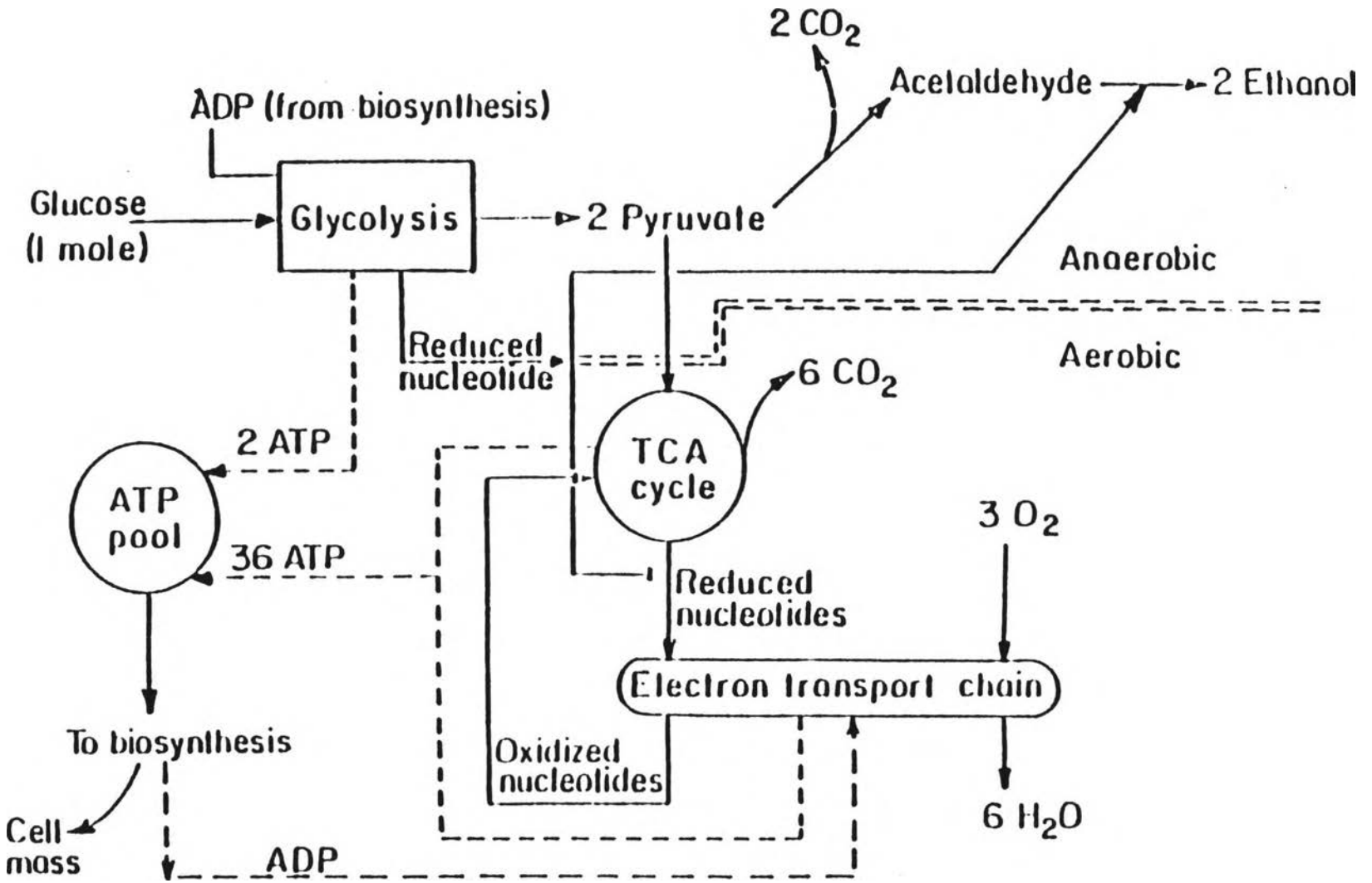


Figure 5. Catabolism of yeasts (30).

Table 1. Ability of *Saccharomyces* to Ferment Sugars

Type of Basic Subunit	Sugar	Basic Unit	*S. cervesiae*	*S. uvarum*
Aldose Sugars	Glucose	Glucose	+	+
	Maltose	Glucose	+	+
	Maltotriose	Glucose	+	+
	Cellobiose	Glucose	-	-
	Trehalose	Glucose	+/-	+/-
	Galactose	Galactose	+	+
	Mannose	Mannose	+	+
	Lactose	Glucose, Galactose	-	-
	Melibiose	Glucose, Galactose	-	+
Ketose Sugars	Fructose	Fructose	+	+
	Sorbose	Sorbose	-	-
Aldoses & Ketoses	Sucrose	Glucose, Fructose	+	+
	Raffinose	Glucose, Fructose Galactose	+/-	+
Deoxy-Sugars	Rhamnose	6-Deoxymannose	-	-
	Deoxyribose	2-Deoxyribose	+/-	+/-
Aldose Sugars	Arabinose	Arabinose	-	-
	Xylose	Xylose	-	-

than 30 g/L), but increases rapidly at higher concentrations with growth generally completely inhibited at ethanol concentrations greater than 120 g/L.

5.3. Zymomonas mobilis

Bacterial ethanol fermentations using strains of Zymomonas mobilis have been found to offer many advantages over traditional yeast fermentations. These include higher specific rates of sugar uptake and ethanol production and improved yields (52). A disadvantage is it can only ferment glucose, fructose, and sucrose via the Entner-Doudoroff pathway and cannot utilize other carbon sources such as maltose and starch (59). The method by which Zymomonas, an anaerobic organism, ferments ethanol is of interest since the Entner-Doudoroff pathway (fig. 6) is normally found only in aerobes.

The pH range and temperature sensitivity for the growth of several species is given by Swings and DeLey (59). In general, growth has been observed over the pH range of 3.5 to 7.9. The optimal temperature range of this bacterium is reported to be between 25 and 31^{o}C. All strains grow at 30^{o}C, but growth above 40^{o}C is rare with no growth reported above 42^{o}C. King and Hossain (27) performed a series of batch fermentations to determine the optimum pH, temperature, and initial glucose concentration with respect to the maximum ethanol production rate. They found the optimum pH for Z. mobilis fermentation was 7.0, but specific ethanol production rate, specific growth rate, and end-of-batch ethanol yield were affected by less than 10 percent in the pH range of 6.0 to 7.5. End-of-batch ethanol yield was maximum and nearly constant between 30 and 37^{o}C but decreased by 24 percent

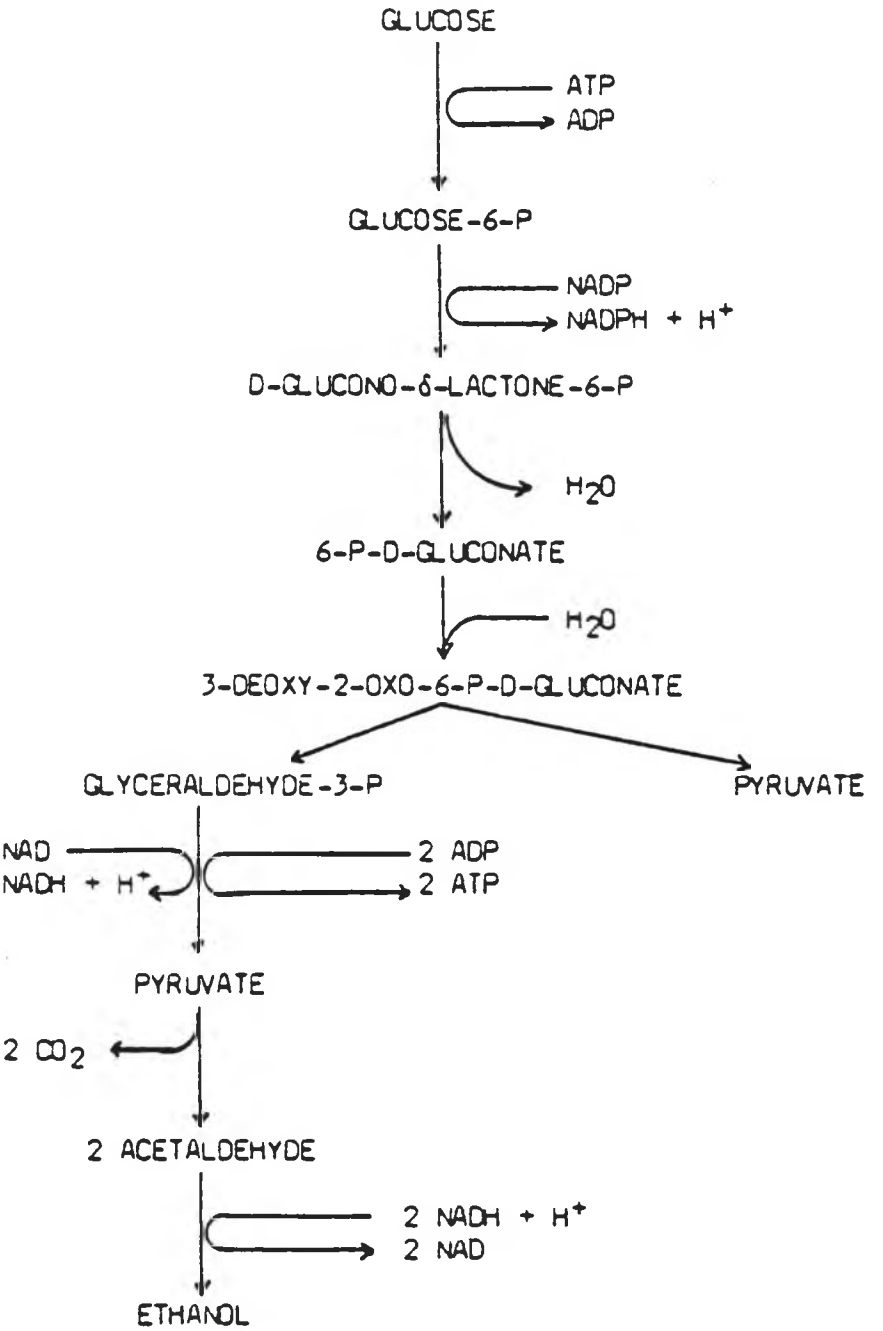


Figure 6. Entner-Doudoroff pathway.

between 37 and 40°C. All other kinetic parameters were greatest at 34°C. The optimum initial glucose concentration of 100 g/L gave the highest ethanol yield with a specific growth rate and ethanol production rate which were less than 10 percent below the maximum observed at 75 g/L.

The major products of the Z. mobilis fermentation are ethanol, carbon dioxide and lactic acid. Glucose metabolism can produce up to 1.8 moles of ethanol per mole of glucose fermented, whereas fructose metabolism can produce up to 1.5 moles of ethanol per mole of fructose fermented (38,59). During fructose metabolism the energetically wasteful products acetaldehyde, glycerol and dihydroxyacetone can be formed (59,62). Traces of acetaldehyde were also reported for glucose metabolism (59). A by-product in the fermentation mixtures of glucose and fructose is sorbitol (62). Zymomonas metabolism of sucrose can lead to the formation of levan (59,62). When yeast extract is used, traces of succinic acid are formed (59). Zymomonas strains are tolerant up to 10 to 15 percent ethanol by volume (7).

MATERIALS AND METHODS

1. Extraction Studies

Sweet sorghum of the Rio variety was collected from USDA plots of Dr. Garry Smith at the Colorado State University (CSU) Agronomy Research Center in Fort Collins, Colorado on September 24, 1984. The material was spread on the floor of the CSU Agricultural and Engineering Research Center to allow wilting and then chopped with an experimental model of a forage chopper three days later. A quantity of 38.5 kg of chopped material with a dry matter content of 43 percent was mixed with 0.16 kg of ensiling inoculum (1 percent on a dry weight basis) containing Lactobacillus plantarum and Streptococcus faecium (Pioneer Hybrid Silabac 1177). The chopped sweet sorghum containing the ensiling inoculum was tightly packed into three 200-L plastic barrels lined with two layers of 4 mil polyethylene bags (Silverstates Plastics, Loveland, CO). Air was displaced by carbon dioxide as a result of sublimation of dry ice placed into the bottom of the container before the chopped sorghum was added. After ten months of storage at 20 to 25^{o}C, the ensiled sweet sorghum was unspoiled.

Extraction experiments were conducted using a pilot scale Bruniche-Olsen continuous countercurrent diffuser (fig. 7) loaned to CSU by the Beet Sugar Development Foundation (Fort Collins, CO). This unit, a model of the continuous slope diffusers typically used in the sugar beet industry, consisted of a round bottomed copper trough (6 inches wide and 4 feet long) containing a single interrupted scroll

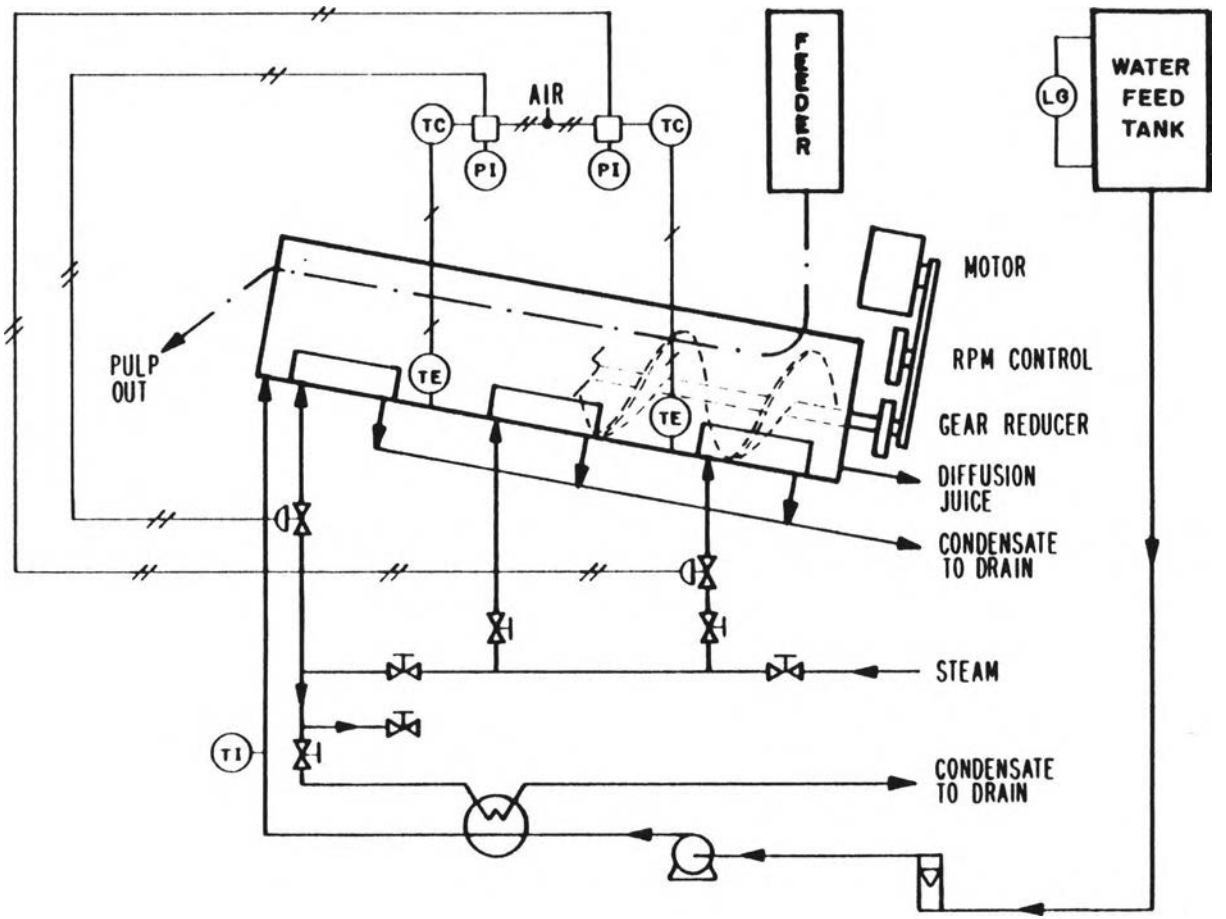


Figure 7. Schematic of the diffuser and auxiliary equipment.

with blades approximately 5.5 inches in diameter. This unit was mounted with a slope of 0.69 degree. Auxiliary equipment included a constant speed belt which delivered ensilage to the lower end of the diffuser and a peristalic pump which fed preheated water into the trough bottom at the higher end of the extraction unit. Diffusion juice exited through a perforated wiped plate at the lower end, while pulp (exhausted sorghum) was collected from a chute at the higher end as it was ejected by the action of a rotating scroll. Temperature was maintained by three jackets, to which the steam flow was controlled by two thermocouples mounted between the three sections of scrolls in the sorghum mass.

The retention time of the sweet sorghum in the diffuser was determined by the time required for a certain amount of ensilage dyed with crystal violet to pass through the unit. Dispersion of the dyed samples occurred because of the mixing action of the scroll during passage from the lower end of the unit to the higher end. The fixed rotation rate of the scroll resulted in a retention time between 90 and 120 minutes.

A set of experiments was performed to determine the effect of temperature and liquid-solid ratio (L/S ratio; the mass of feed water to the mass of feed ensilage) on the fermentable sugar recovery. The temperatures studied were 50 and 70^{o}C and L/S ratios were varied between 5/1 and 1/1 for each temperature.

At the start of runs for a given temperature, 0.6 kg of sweet sorghum ensilage was placed in the diffuser and water was added until overflow appeared in the juice sample tube at the lower end of the unit. The mixture was then heated to the temperature set point. When

the desired temperature was reached, feed was then started by placing 0.6 kg of sorghum on 1.22 meters (4 ft) of the constant speed belt, which corresponded to a solid feedrate of 26.3 g/min. For a given L/S ratio, when no change in the dissolved solids content of the diffusion juice was observed (using a Bausch and Lomb Abbe-3L refractometer) over a one hour period, steady state was assumed. Juice and pulp samples were then collected in tared containers for a specified amount of time. Representative samples of the feed ensilage, pulp, and juice were frozen at -3^{o}C and later analyzed for moisture content, and sugar and organic acid concentrations by High Performance Liquid Chromatography (HPLC) analysis as described below. After sampling, the L/S ratio was changed by altering the water feedrate.

Runs were conducted during four days in 1985: 8/20, 8/21, 8/22, and 8/23. For the first two days the temperature set point was 50^{o}C, and for the second two days it was 70^{o}C. On day 8/20 the temperature at the lower end of the diffuser, where the ensilage was fed in, was 41 $\pm$ 5^{o}C, while at the high end of the diffuser, where the exhausted material bunched up before being ejected by the rotating scroll, the temperature was 64 $\pm$ 3^{o}C. On day 8/21, the low end diffuser temperature was 45 $\pm$ 3^{o}C, whereas the high end of the diffuser was 61 $\pm$ 4^{o}C. The temperature variation across the rest of the diffuser for both days (8/20 and 8/21) was 50 $\pm$ 5^{o}C. For the 70^{o}C runs, the temperature at the low end of the diffuser was 62 $\pm$ 4^{o}C for day 8/22 and 59 $\pm$ 4^{o}C for day 8/23. The temperature at the high end of the diffuser was 69 $\pm$ 3^{o}C and 63 $\pm$ 2^{o}C for day 8/22 and 8/23, respectively. The temperature variation for the rest of the diffuser on both days was 70 $\pm$ 5^{o}C.

The temperatures below the set point at the low end of the diffuser were due to fresh ensilage being fed into the diffuser at room temperature. Temperatures at the high end of the diffuser were higher than the set point for the 50°C runs and lower than the set point for the 70°C runs. However, the temperatures at the high end of the diffuser were all similiar. This was due to the temperature of the water staying within the range of 164 to 169°C for all four days while being fed into the bottom of the high end of the diffuser .

Recall the L/S ratio was changed by altering the water feedrate. For each of the four days, during the runs, the solid feedrate was 26.3 ± 0.1 g/min. After changing to a new L/S ratio, there was no fluctuation in the rotameter reading which implied a steady flow rate of water. Therefore, very little fluctuation in the magnitude of the L/S ratio occured at a given L/S ratio because the solid feedrate and water flow rate varied slightly.

2. Concentration of Diffusion Juice Samples

Juice from the extractor did not have a fermentable sugar concentration suitable for fermentation work as determined by refractometry and preliminary HPLC analysis for glucose and lactic acid concentrations. Therefore, the juice was concentrated using a Buchi R110 rotary evaporator at 50°C until the juice contained at least 8 percent dissolved solids. The concentrated juice obtained from all extraction runs at each temperature were combined and mixed to obtain uniform concentrations of sugars and organic acids. The mixed concentrated juice was used in the subsequent fermentation studies.

The diffusion juice and concentrated juice were stored at -3°C for the time periods between the various analyses and operations.

3. Fermentation Studies

Batch fermentations of one liter working volume were conducted using a 2 liter capacity Marubishi Model MD series bench top fermenter. Temperature and agitation control were provided by the Marubishi unit, while pH control was provided by a B. Braun Instruments Model M measurement and regulation system using an Ingold sterilizable electrode.

Fermentations were conducted for three organisms: 1) Clostridium acetobutylicum (ATCC 824), 2) Saccharomyces uvarum (NRRL Y-1347), and 3) Zymomonas mobilis (ATCC 10988). The C. acetobutylicum fermentations were performed under anaerobic conditions at 37°C with a 100 rpm agitation rate. The pH was controlled at 5.0, after dropping from an initial value of 6.5. In one case the pH did not drop to 5, and it was forced down by the pH regulation system. The S. uvarum fermentations were performed under anaerobic conditions at 30°C and pH 5 with a 100 rpm agitation rate. The Z. mobilis fermentations were performed under anaerobic conditions at 31°C and pH 6.2 with a 100 rpm agitation rate. Anaerobic conditions within the fermenter were created by bubbling sterile nitrogen gas through the medium for a few hours. These three organisms were used for fermentation of both 50°C and 70°C extracted juice concentrates. During each 96 hour fermentation, samples were taken periodically to determine changes in the substrate and product concentrations using the HPLC analysis described below.

Previous experience with sweet sorghum juice fermentations indicated that an adaption procedure was necessary as a preliminary step to fermentation. This was due to poor growth and product yields when sweet sorghum juice was inoculated with organisms grown with standard medium such as yeast or MIT medium (table 2). Attempts were made to use the adaption procedure from a previous study (35). Figures 8 to 9 are schematics of this adaption procedure. It was found, for the present work, that this adaption procedure had long lag times and periodically the microbes would not grow past a 50/50 (v/v) medium/sorghum juice mixture. Therefore, the following inoculation procedure listed in Figures 10 to 12, was developed. As the tables indicate, the fermenter contained sweet sorghum concentrated juice with additional minerals. At approximately the same time the fermenter was inoculated, a test tube with 10 mls of sweet sorghum concentrated juice with no additional minerals was inoculated (inoculum size for both the fermenter and test tube was 10 percent (v/v)) and placed into an anaerobic chamber. The test tubes had lag times longer than the fermenter, approximately 24 to 48 hours longer, or showed no growth at all. Thus, the need for additional minerals was essential.

Media used in the inoculation schemes (table 2) were sterilized for 15 minutes at 121^{o}C, whereas sorghum juice with additional salts (salts were added directly to sweet sorghum juice before autoclaving) was sterilized for 15 minutes at 100^{o}C. Linden et al. (32) have shown that heating a 10:1 water:ensilage mixture (70 percent moisture) at 80^{o}C for 15 minutes inactivated 99.9 percent of the ensilage borne organisms. The autoclave utilized in this study was not capable of pasteurization cycles. The lowest temperature it would achieve was

Table 2. Media Used in Adaption and Inoculation Procedures

Component	Concentration (g/L)
MIT Medium Used for *Clostridium acetobutylicum*	
$MnSO_4$	0.01
$FeSO_4$	0.01
$MgSO_4$	0.20
Cystein	0.50
KH_2PO_4	0.75
K_2HPO_4	0.75
NaCl	1.00
Asparagine	2.00
$(NH_4)_2SO_4$	2.00
Yeast Extract	5.00
pH Medium to 6.5	
Yeast Medium Used for *Saccharomyces uvarum*	
$CaCl_2$	0.28
KH_2PO_4	0.50
$MgSO_4$	0.50
Sodium citrate	1.26
Na_2HPO_4-$7H_2O$	3.90
Citric Acid	4.30
NH_4Cl	8.00
Yeast Extract	8.00
Glucose	18.20
pH Medium to 5.0	
Zm Medium Used for *Zymomonas mobilis*	
$MgSO_4$-$7H_2O$	0.50
KH_2PO_4	1.00
$(NH_4)_2SO_4$	1.00
Yeast Extract	3.00
Glucose*	50.00
pH Medium to 6.2	

* Sterilized separately.

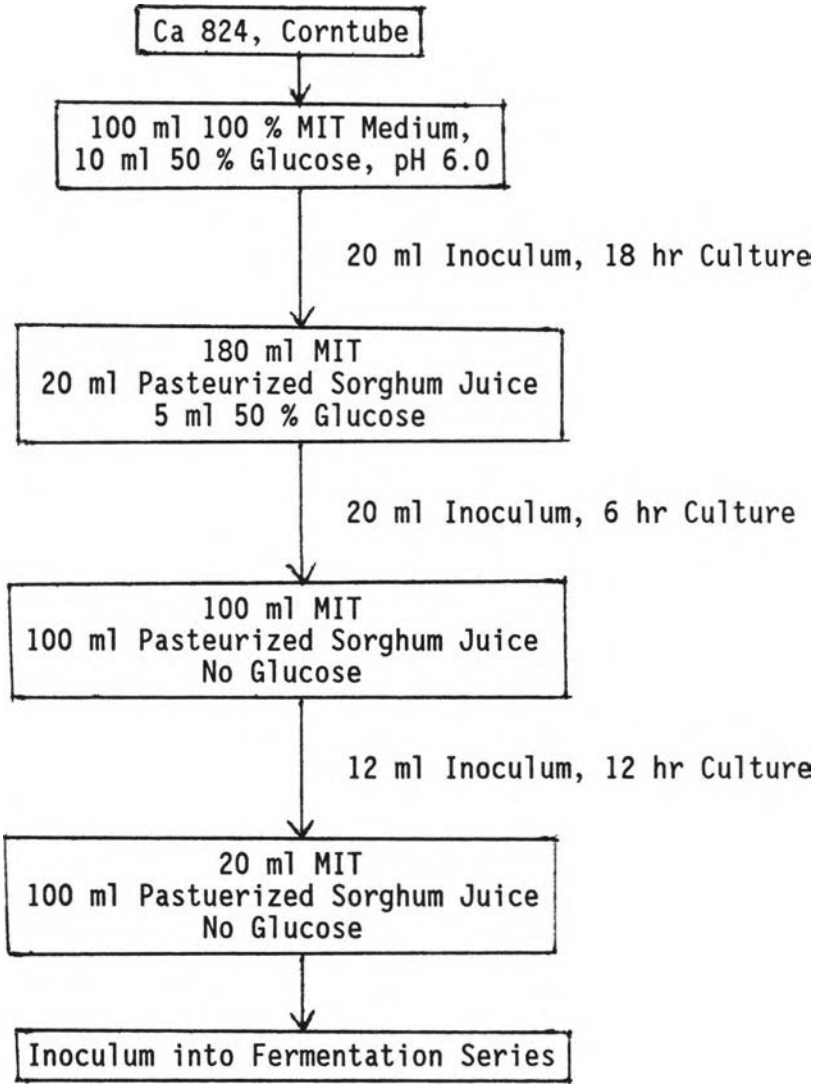


Figure 8. Adaption scheme for Clostridium acetobutylicum fermentations. Sorghum juice was pastuerized at 70°C for 60 minutes. Bench top experiments were performed under anaerobic conditions at 37°C.

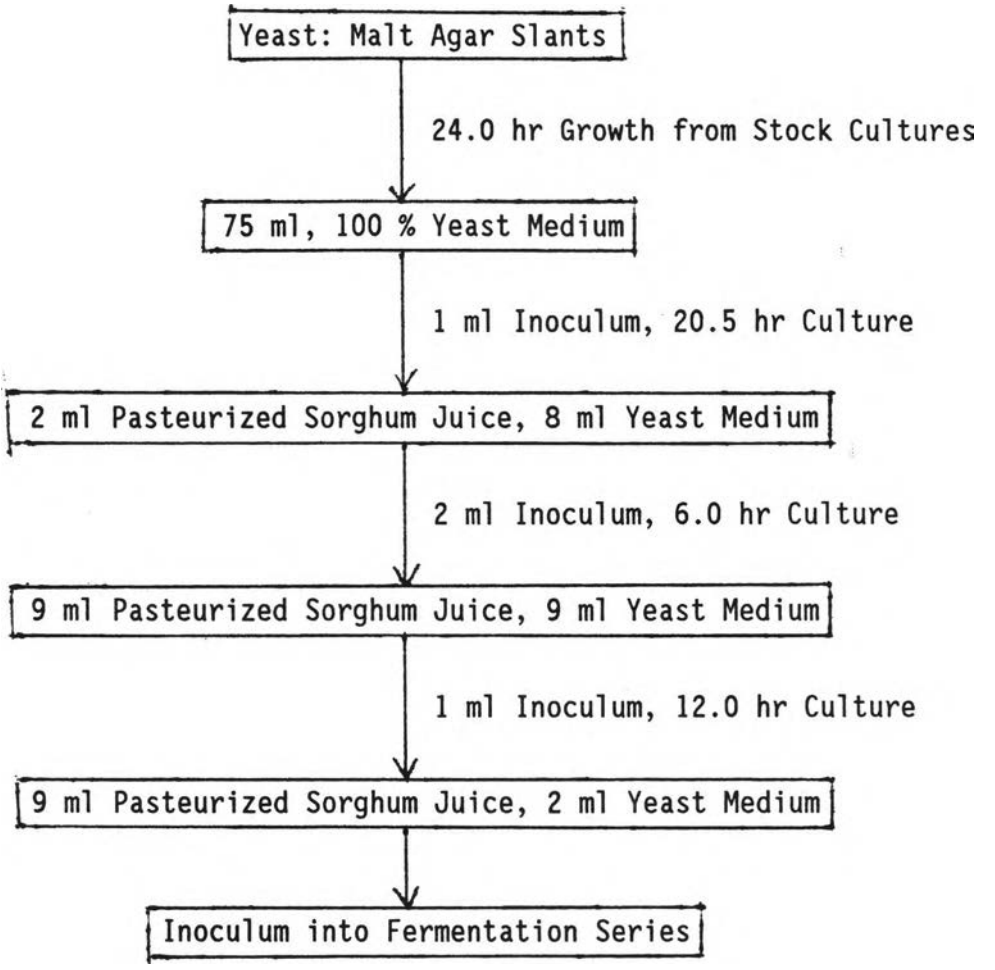
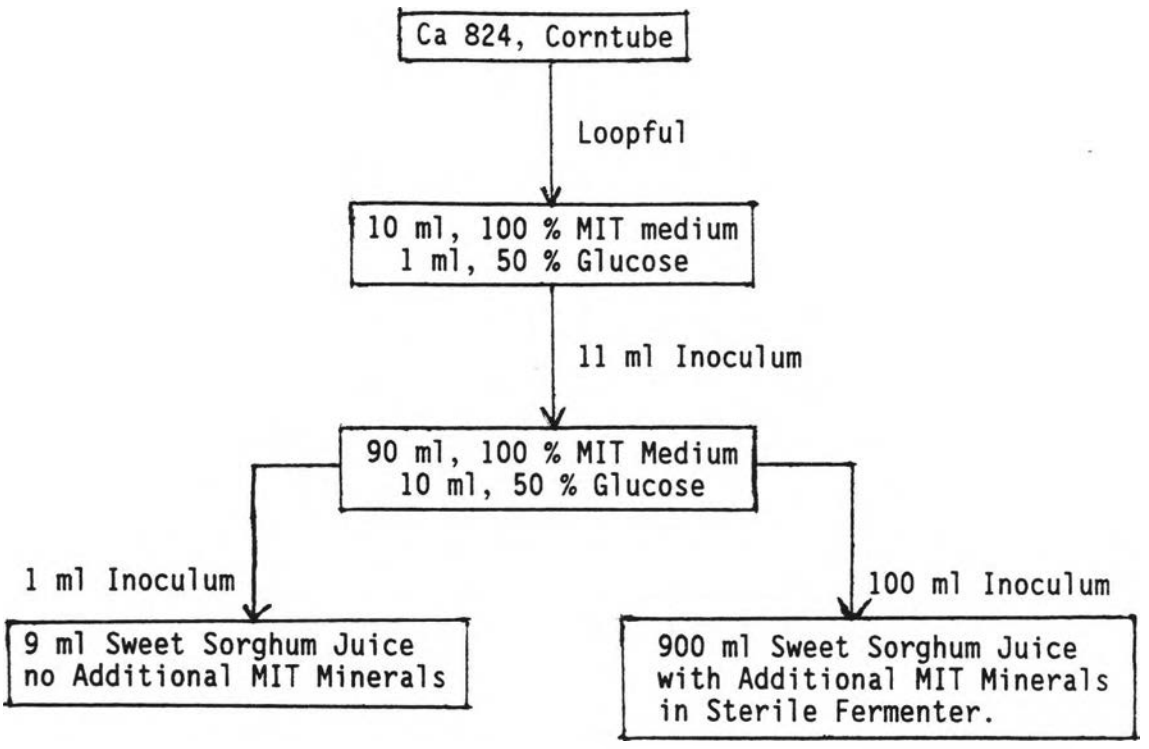


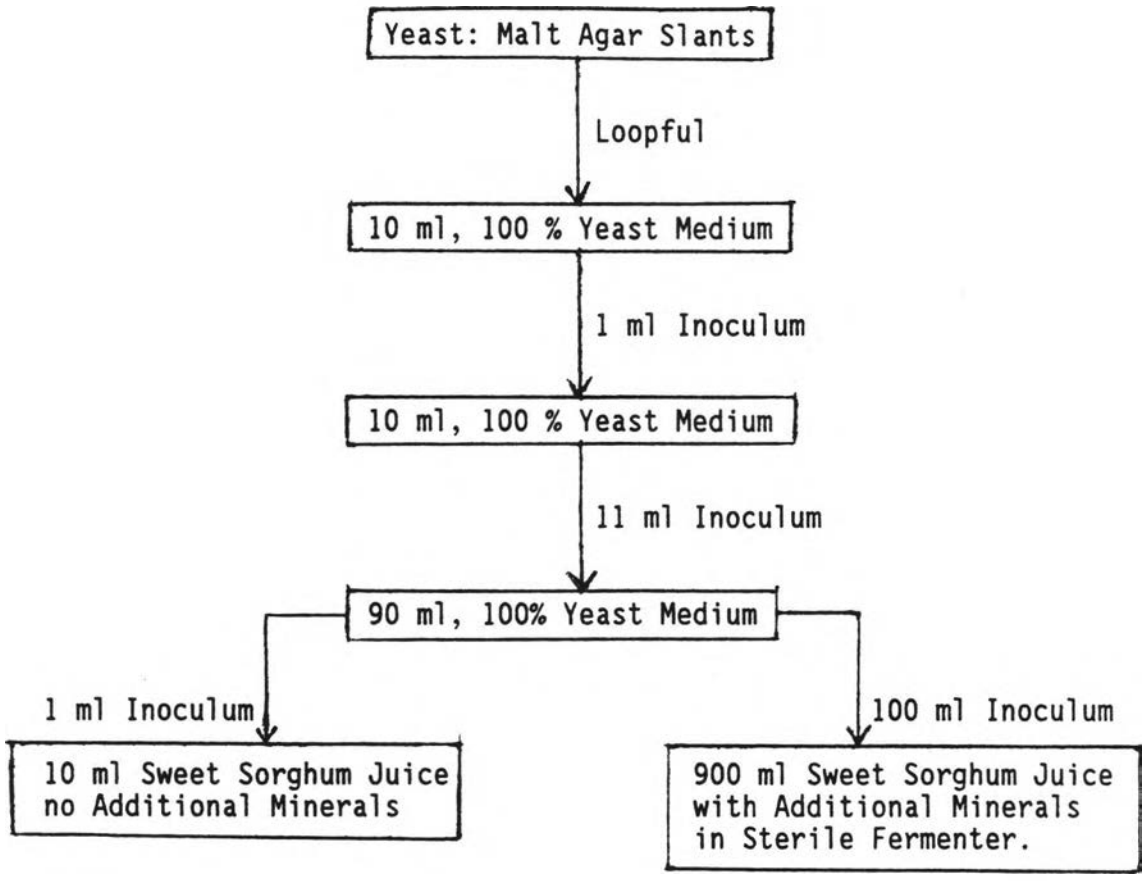
Figure 9. Adaption scheme for Sacchromyces uvarum fermentations. Sorghum juice was pasteurized at 70°C for 60 minutes. Bench top experiments were performed under anaerobic conditions at 30°C.



Additional Minerals

Component	Concentration (g/L)
$MnSO_4$	0.01
$FeSO_4$	0.01
$MgSO_4$	0.20
Cystein	0.50
KH_2PO_4	0.75
K_2HPO_4	0.75
NaCl	1.00
Asparagine	2.00
$(NH_4)_2SO_4$	2.00
Yeast Extract	5.00

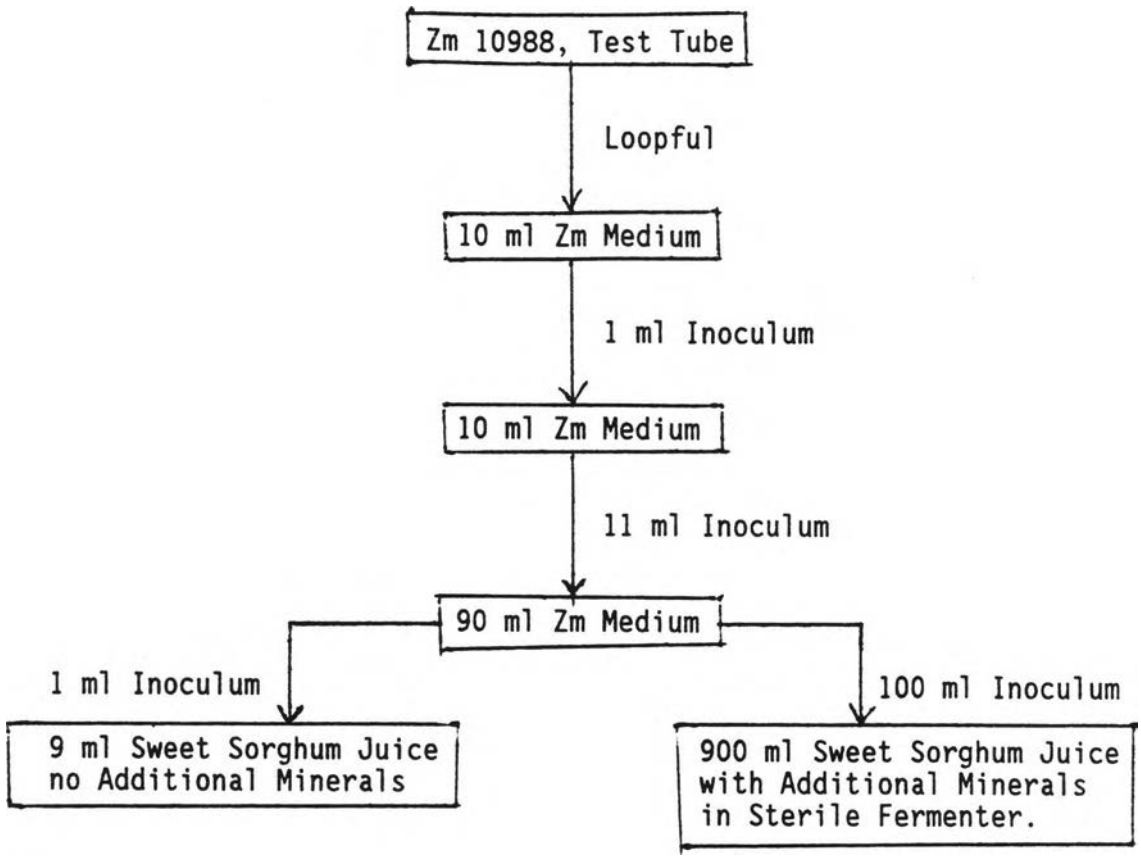
Figure 10. Inoculation scheme for Clostridium acetobutylicum fermentations. Medium was sterilized for 15 minutes at 121°C and the sorghum juice with additional minerals for 15 minutes at 100°C.



Additional Minerals

Component	Concentration (g/L)
$CaCl_2$	0.28
KH_2PO_4	0.50
$MgSO_4$	0.50
Na_2HPO_4 $7H_2O$	3.90
NH_4Cl	8.00
Yeast Extract	8.00

Figure 11. Inoculation scheme for Sacchromyces uvarum fermentations. Medium was sterilized for 15 minutes at 121°C and the sweet sorghum juice with additional minerals for 15 minutes at 100°C.



Additional Minerals

Component	Concentration (g/L)
$MgSO_4$ $7H_2O$	0.50
KH_2PO_4	1.00
$(NH_4)_2SO_4$	1.00
Yeast Extract	3.00

Figure 12. Inoculation scheme for Zymomonas mobilis fermentations. Medium was sterilized for 15 minutes at 121°C and the sorghum juice with additional minerals for 15 minutes at 100°C.

100°C. Therefore, the sweet sorghum juice was autoclaved for 15 minutes at 100°C instead of 80°C. The inoculation operations, with exception of the fermentations, were carried out in an anaerobic chamber (COY Products, Ann Arbor, MI) containing an atmosphere of 85 percent nitrogen, 10 percent hydrogen and 5 percent carbon dioxide.

4. Analytical Methods

4.1. Moisture Content

Samples were dried in an air convection oven at 100°C for 24 hours. The difference between the initial and final weights was used to calculate a percent moisture content.

4.2. Dissolved Solids

Dissolved solids (Brix) were determined using a Bausch and Lomb Abbe-3L refractometer with temperature regulation maintained by a 20°C recirculating water bath. The instrument was calibrated by comparing the refractive index reading of 0.0, 1.0, and 10.0 percent (v/v) sucrose solutions to the International Scale of Refractive Indices of Sucrose Solutions at 20°C.

4.3. Fermentable Sugars, Organic Acids, and Solvents Concentrations

Samples from the extractions and fermentations were analyzed using a Waters Associates Model 6000-A High Performance Liquid Chromatograph with a Bio-rad (Richmond, CA) organic acid column (HPX-87H, 300 x 7.8 mm). Twenty microliters of a sample were eluted through the 47°C column by 0.008 N sulfuric acid at a flowrate of 0.6 ml/min, which corresponded to a system pressure of 1000 psig. A Waters Associates

Series R-401 differential refractometer was used to detect peaks while a Waters Associates Data Module, Model 730, integrator was used to quantify peaks.

4.4. Preparation of Samples

The solid samples of ensilage and pulp were prepared for analysis by two methods, an expression method, and a blended extraction method. For the expression method, material was packed into a 60 ml plastic syringe and the juice pressed out using a Carver laboratory press. Figure 13 shows the procedure for the blended extraction method. This procedure required sufficient water to liquefy the solid samples. The water made the pulp samples too dilute to measure by High Performance Liquid Chromatography (HPLC). Therefore, the expression method was used to prepare a liquid sample from solid materials for analysis.

The liquid samples from solid materials and the diffusion juice samples were prepared for HPLC analysis by first centrifuging 4 minutes in an Eppendorf microcentrifuge and then filtering through 0.45 micron Millipore filters.

4.5. Specific Gravity Determination

Specific gravity was determined using a Mettler DMA 35 Density Meter. The instrument was calibrated by comparing the specific gravity of water at room temperature to tabulated specific gravity values of water at various temperatures provided by Mettler. Specific gravity was determined for the diffusion juice samples and the juice from the expression and blended extraction method preparation(s) of the ensilage and pulp samples.

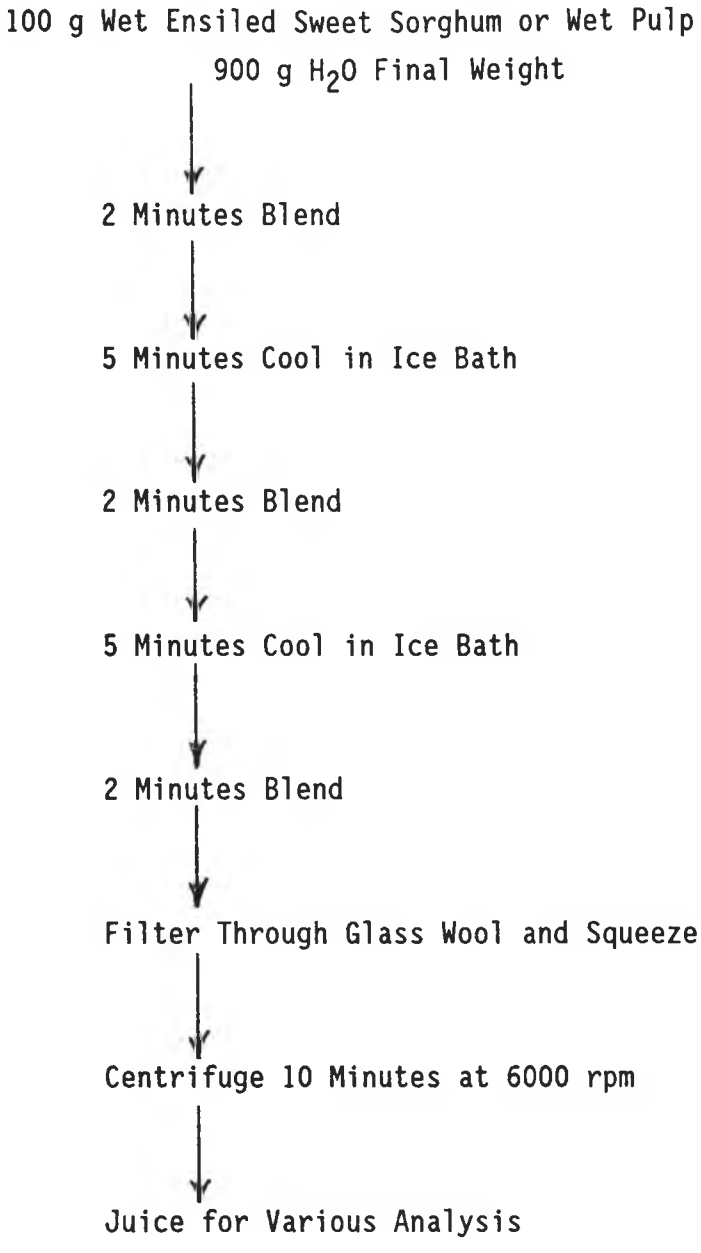


Figure 13. Procedure for Preparation of Solid Samples by the Blended Extraction Method.

RESULTS AND DISCUSSION

1. Extraction Studies

The results and discussion for the extraction studies will be presented as follows: analysis of the sweet sorghum ensilage, the effect of liquid-solid ratio on the pulp component concentrations, the effect of liquid-solid ratio on the juice component concentrations, the effect of liquid-solid ratio on the extraction efficiencies, the analysis of an 8 hour run at the 70^{o}C 1.5 liquid-solid ratio, followed by a model that will predict the pulp component concentrations (and therefore the extraction efficiencies) from the known sweet sorghum component concentrations.

Table 3 shows the content of sugars and organic acids expressed from ensiled sweet sorghum. The table lists the components as they would appear on a typical High Performance Liquid Chromatography (HPLC) chromatogram of an expressed sample (fig. 14). Oligosaccharides, identified as stachyose/raffinose, represented two individual peaks which were quantified as one peak using the response factor for stachyose. Sucrose/unknown #1 represented two individual peaks, sucrose and unknown #1, quantified together using the response factor for sucrose. Fructose/arabinose represented one peak which included fructose, arabinose, mannitol, xylose and other possible pentoses. This peak was quantified by using the response factor for fructose. There was no evidence that other peaks listed in Table 3 contained any component except that identified. The sugars sucrose, glucose, and

Table 3. Composition of Sweet Sorghum Ensilage Feed to Extractor*

Component (mg/g dry wt)	Day 8/20 Silo #2 Sample FE 8/20	Day 8/21 Silo #2 Sample FE 8/21	Day 8/22 Silo #1 Sample FE 8/22	Day 8/23 Silo #1 Sample FE 8/23
Stachyose/Raffinose	32.37	41.24	24.82	27.18
Sucrose/Unknown #1	12.51	15.56	12.90	7.33
Glucose	24.11	23.51	21.33	11.45
Fructose/Arabinose	93.86	119.77	78.69	98.61
Lactic Acid	77.69	90.71	67.73	79.81
Acetic Acid	19.12	25.67	17.05	21.21
Unknown #2	3.25	4.11	1.57	1.62
Propionic Acid	5.56	6.97	2.77	3.93
Ethanol	23.99	29.67	18.75	28.27
Total	292.57	357.21	245.62	279.40

* Composition values are averages from duplicate analysis of three samples. Stachyose/Raffinose represents oligosaccharides. Sucrose/Unknown #1 represents two separate peaks, sucrose and unknown #1, quantified as sucrose. Fructose/Arabinose represents one peak which included fructose, arabinose, mannitol, xylose, and other possible pentoses.

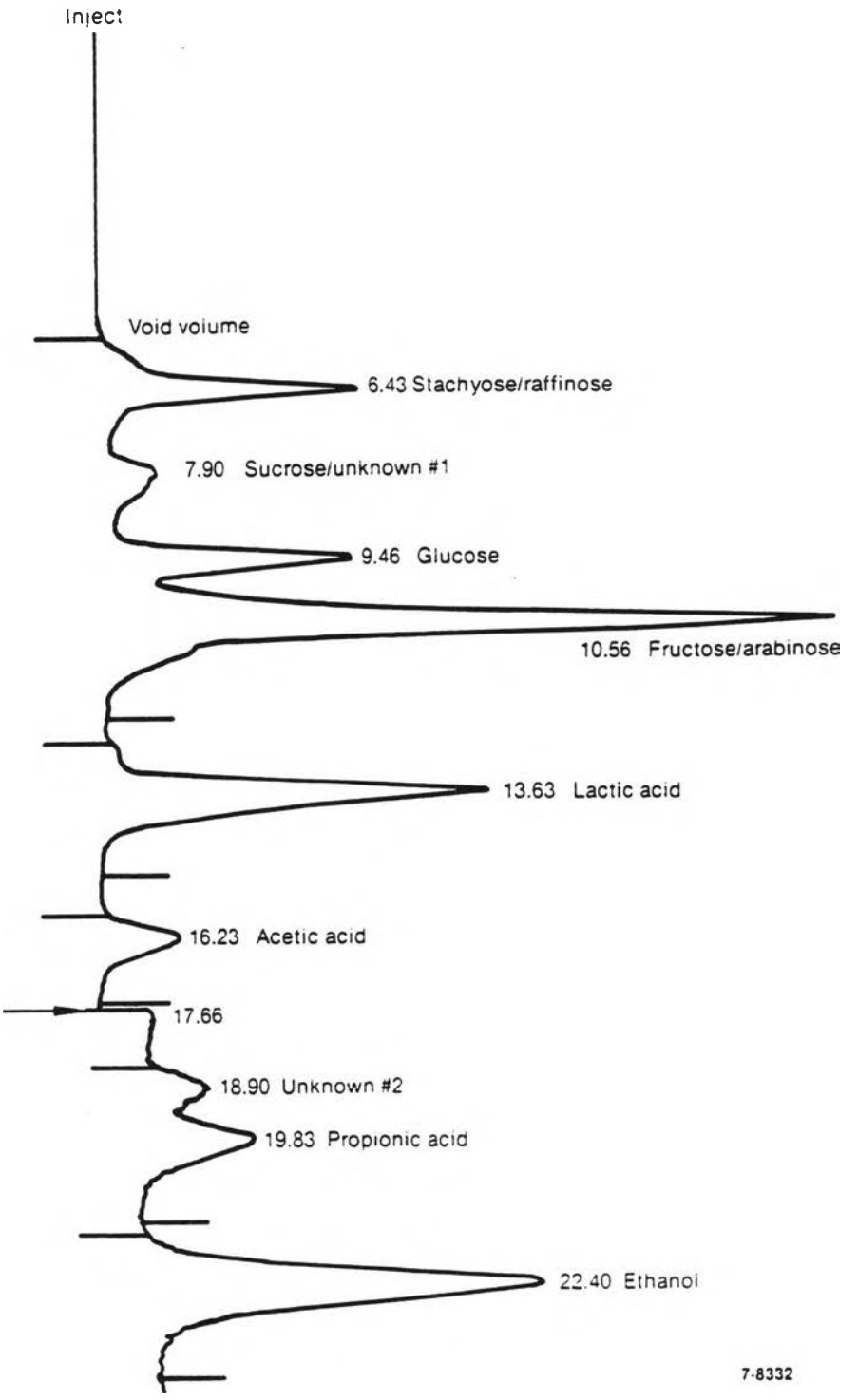


Figure 14. A typical High Performance Liquid Chromatography chromatogram of an expressed sample.

fructose were natural components of the sweet sorghum. The oligosaccharides, quantified as stachyose, may have come from starch depolymerization from acid hydrolysis, as mentioned earlier. Xylose and arabinose came from the acid hydrolysis of hemicellulose. Mannitol resulted from the metabolism of fructose by lactic acid bacteria. Lactic acid, acetic acid, propionic acid, and ethanol were from the Lactobacillus metabolism of glucose. Butyric acid was not detected in the sweet sorghum ensilage indicating there was no clostridia activity in the ensilage, implying there was favorable ensiling conditions.

There were two components which could not be identified. Unknown #1 eluted between sucrose and glucose and was quantified with the sucrose peak. This peak could possible be cis-aconitic acid, which was reported by Kubadinow (29) to have a retention time between that of sucrose and glucose with the same chromatographic system as used in these studies. Also, as mentioned earlier, aconitic acid was a component of sweet sorghum. The other unknown component, unknown #2, eluated between acetic acid and propionic acid. It was quantified by using the response factor for propionic acid. This unknown could be one of two things. It could be a component of sweet sorghum because it also showed up in chromatograms of non-ensiled sweet sorghum extracts (22) or a plasticyin, a component of the polyethylene bags used to store the sweet sorghum in. The non-ensiled sweet sorghum was also stored in these bags. A component(s) of the plastic bags could have leached into the sorghum due to the acidic environment caused by the ensiling conditions. The plasticyin also may have been loosely bound on the inside of the bags, as the bags were not washed prior to packing them with sweet sorghum.

The ensilage for the extraction studies came from two different silos, which were plastic barrels lined with polyethylene bags packed with inoculated sweet sorghum. On the first two days, 8/20 and 8/21, ensilage was taken from one silo (silo 2) for extraction studies; and on the last two days, 8/22 and 8/23, it came from the other silo (silo 1). The difference in origin helped explain the difference in concentration between the first two days and the last two days, which are so labeled across the top of Table 3. Another trend was that the respective second day samples, 8/21 and 8/23, had a higher total percentage of components than the first day samples from the same silo. This could be due to cell sap migration from the top to the bottom of the silo. As shown in Table 4, the moisture content of the ensilage was higher at the bottom of the silo than at the top. This gives creditability to the fact that cell sap containing sugars and organic acids migrated from the top of the silo to the bottom in the time course of the ten month study.

Initially, solid samples such as the ensilage and pulp, were prepared for analysis by extracting them with water in a blender (800 g distilled water to 100 g wet sorghum). This method made the pulp and pressed pulp samples too dilute to measure by HPLC, because sufficient water had to be added to the blender to liquefy the solid samples (at least 6 parts water to 1 part wet pulp were required to liquefy samples). Therefore, the expression method was used to prepare a liquid sample from solid materials for analysis.

Figures 15A to 15E compare the composition of the ensilage which were determined by the blender extraction and expression methods. In general, as Figures 15A to 15D show, the expression method compared

Table 4. Moisture Contents of
Extraction Pulp and Ensiled Sweet Sorghum

Extraction Pulp

Day	Run	L/S Ratio	Temperature (^{o}C)	Moisture (%)
1	1	5.0	50	83.0 ± 0.6
	2	4.5		82.9 ± 0.9
	3	4.0		82.3 ± 2.6
2	4	3.5		82.8 ± 1.1
	5	2.5		84.5 ± 0.6
3	6	5.0	70	86.1 ± 0.1
	7	4.0		83.5 ± 0.3
	8	3.0		83.6 ± 1.0
	9	2.0		84.7 ± 0.4
4	10 A-E	1.5		83.8 ± 0.6
	10 F-J	1.5		83.7 ± 0.7

Sweet Sorghum Ensilage

Day	Silo	Moisture (%)
1	2	73.2 ± 0.3
2	2	75.6 ± 0.2
3	1	68.6 ± 1.5
4	1	74.7 ± 1.3

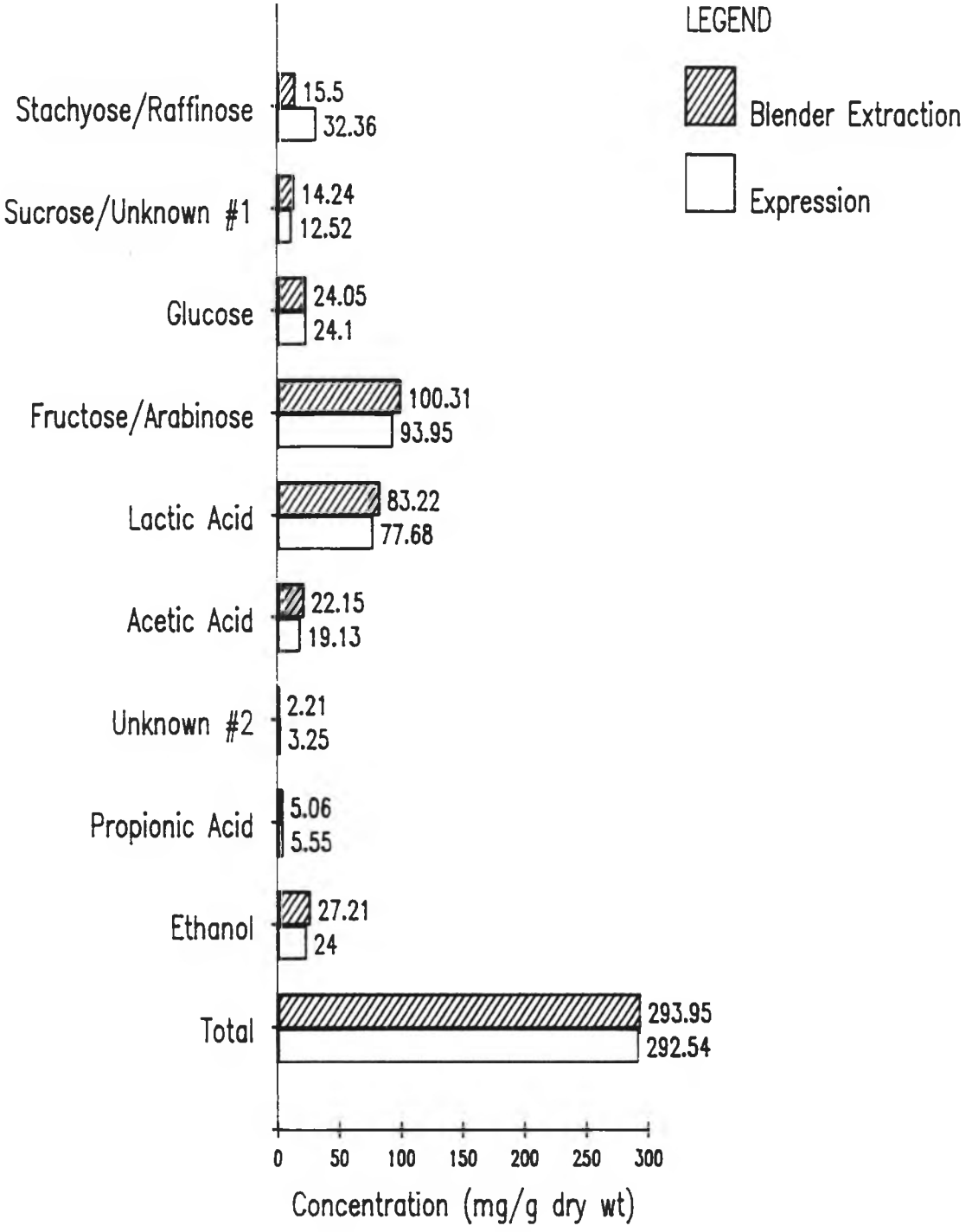


Figure 15A. Comparison of blender extraction to expression method for sweet sorhgum ensilage composition for sample FE 8/20.

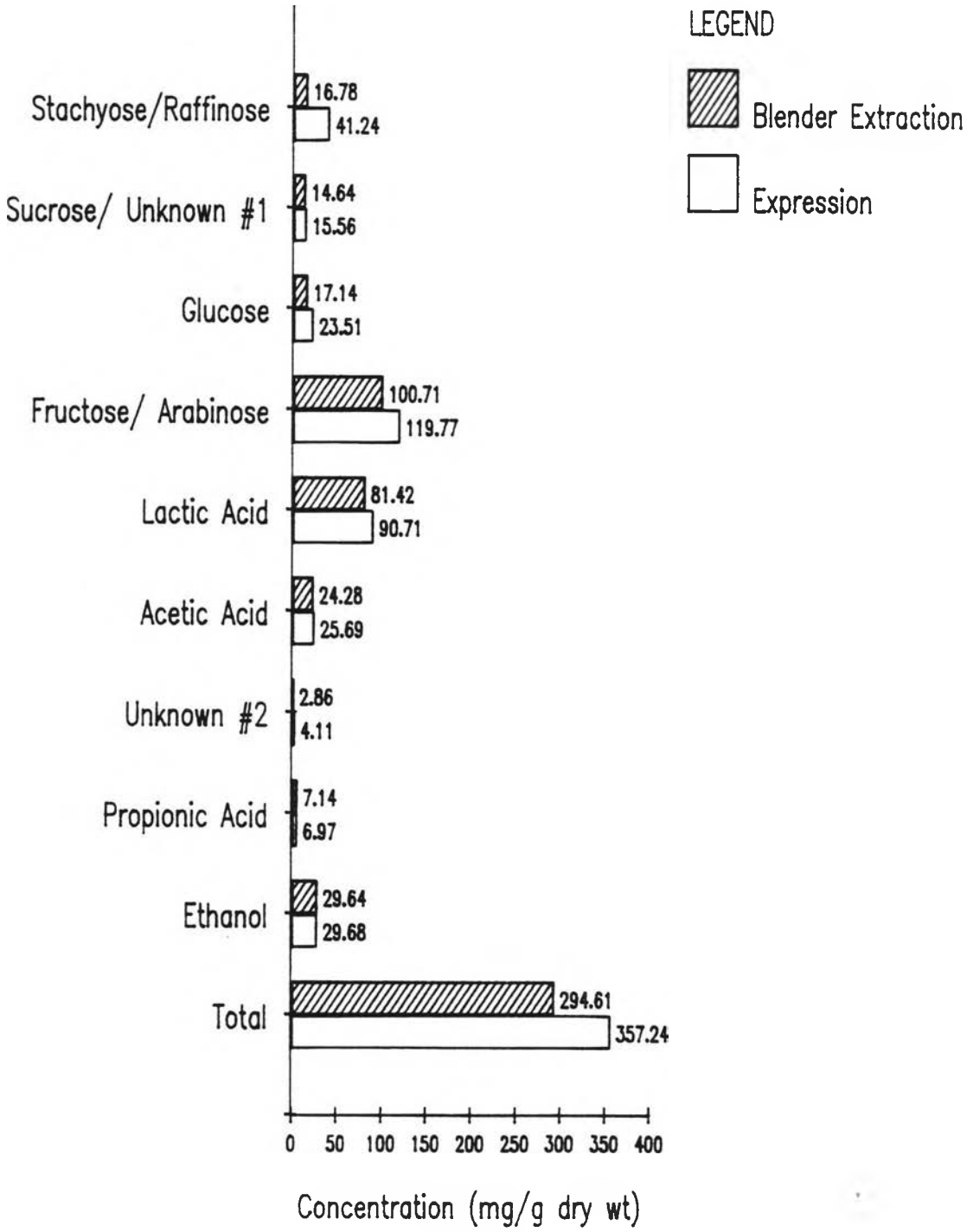


Figure 15B. Comparison of blender extraction to expression method for sweet sorhgum ensilage composition for sample FE 8/21.

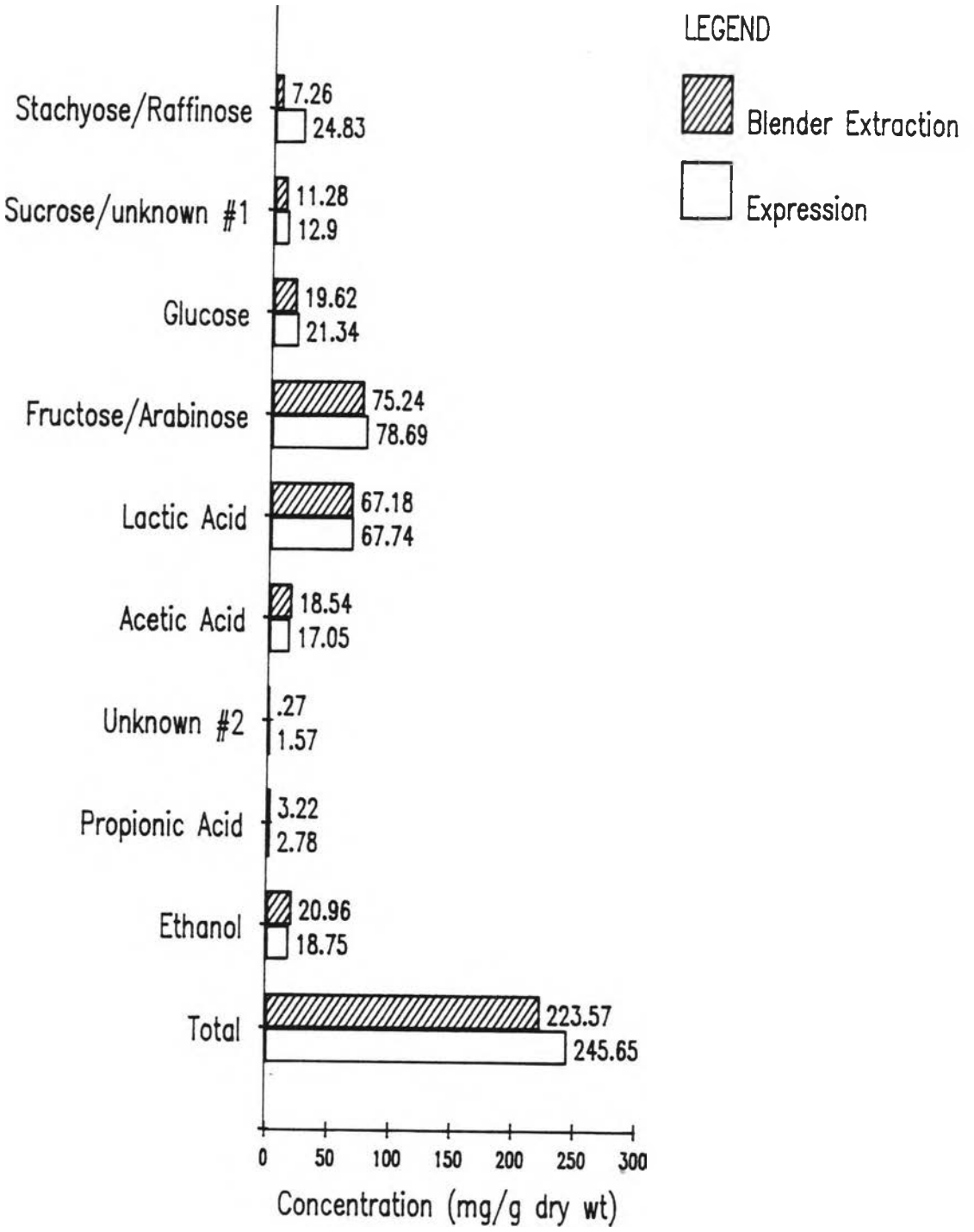


Figure 15C. Comparison of blender extraction to expression method for sweet sorhgum ensilage composition for sample FE 8/22.

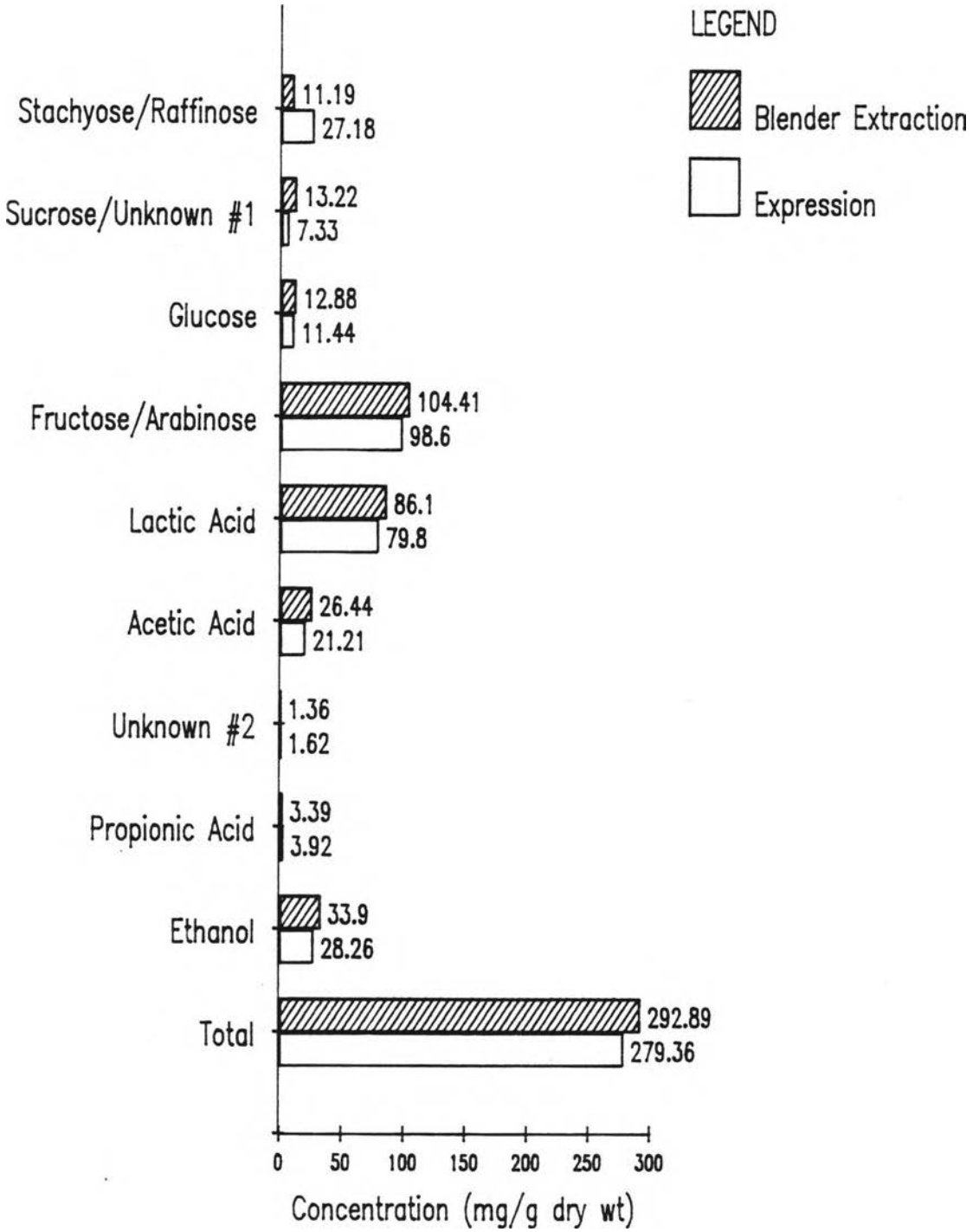


Figure 15D. Comparison of blender extraction to expression method for sweet sorhgum ensilage composition for sample FE 8/23.

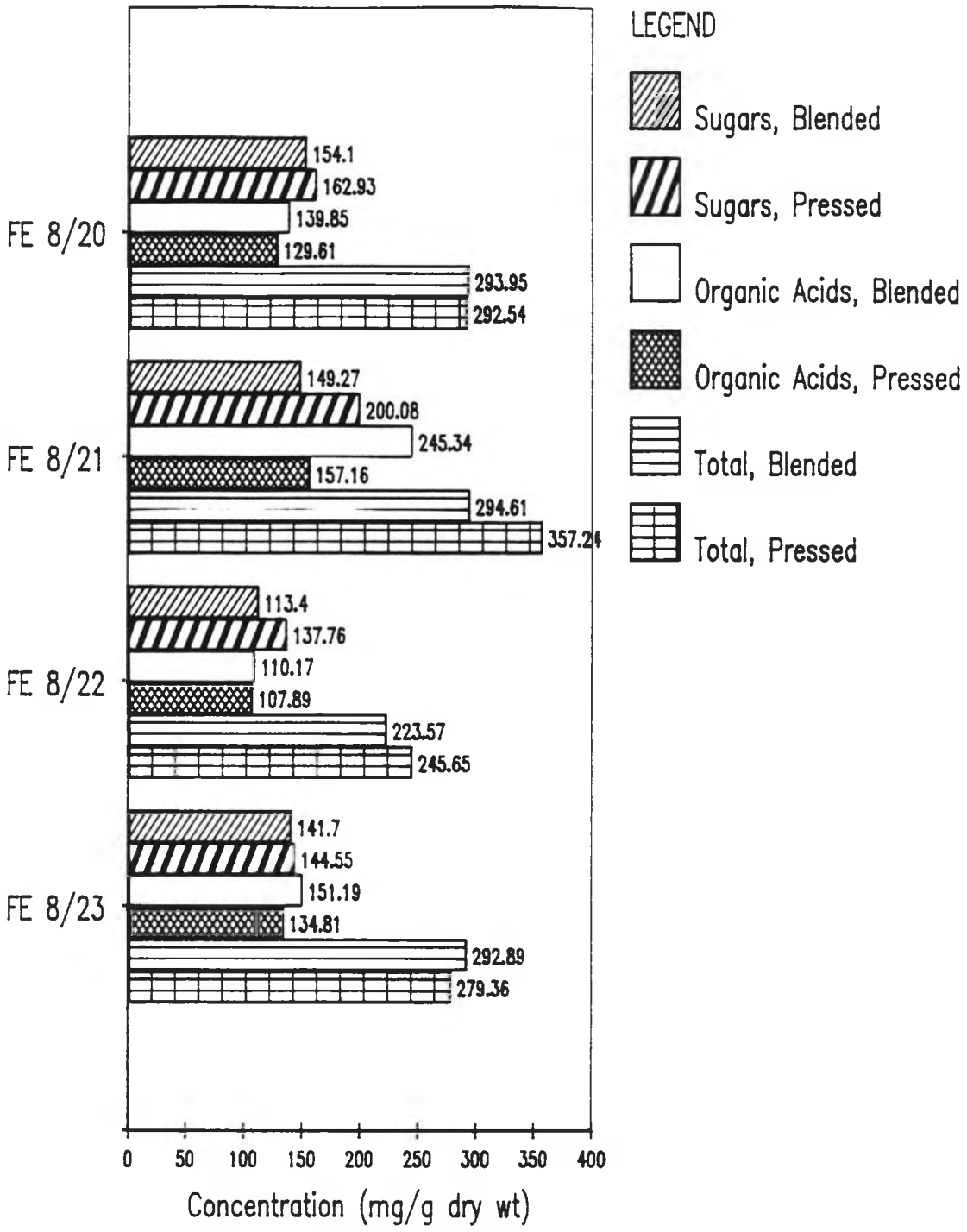


Figure 15E. Comparison of blender extraction to expression method for sweet sorhgum ensilage composition grouped as sugars and organic acids.

closely with the blender extraction method except that the stachyose/raffinose peak was consistently higher in the expression samples. This may be due to the large oligosaccharides having a sufficiently low solubility or diffusivity that they were not extracted by the blender extraction method. By grouping the components of the sweet sorghum as sugars and organic acids, Figure 15E shows that the two extraction methods gave similar results. The exception was the sugars for samples FE 8/21 and FE 8/22, where the majority of the difference came from the stachyose analysis.

Figures 16 and 17 show the concentration for each soluble component in the extracted pulps obtained from the continuous countercurrent 50°C and 70°C runs respectively. The other variable investigated during these runs was liquid-solid (L/S) ratio. The pulp component concentrations were determined with liquid obtained by expression of samples. As the L/S ratio was decreased, the concentrations of soluble components in the pulp samples increased. This would result in a diminished concentration gradient between solid and liquid phases. The difference would cause lower rates of diffusion to occur at the lower L/S ratios. As Figures 16 and 17 show, this was the case for each component with a few exceptions. The first exception would be the low values for the 50°C, 3.5 L/S ratio study and the low 70°C, 1.5 L/S ratio values. Both of these sets of data were obtained from runs at the start of a new day. Samples were taken when the system was supposedly at equilibrium, based on refractometer readings of diffusion juice samples. However, based on the results of a later study, which will be discussed later, the system may not have been at steady state. This non-equilibrium probably caused the low values at the start of

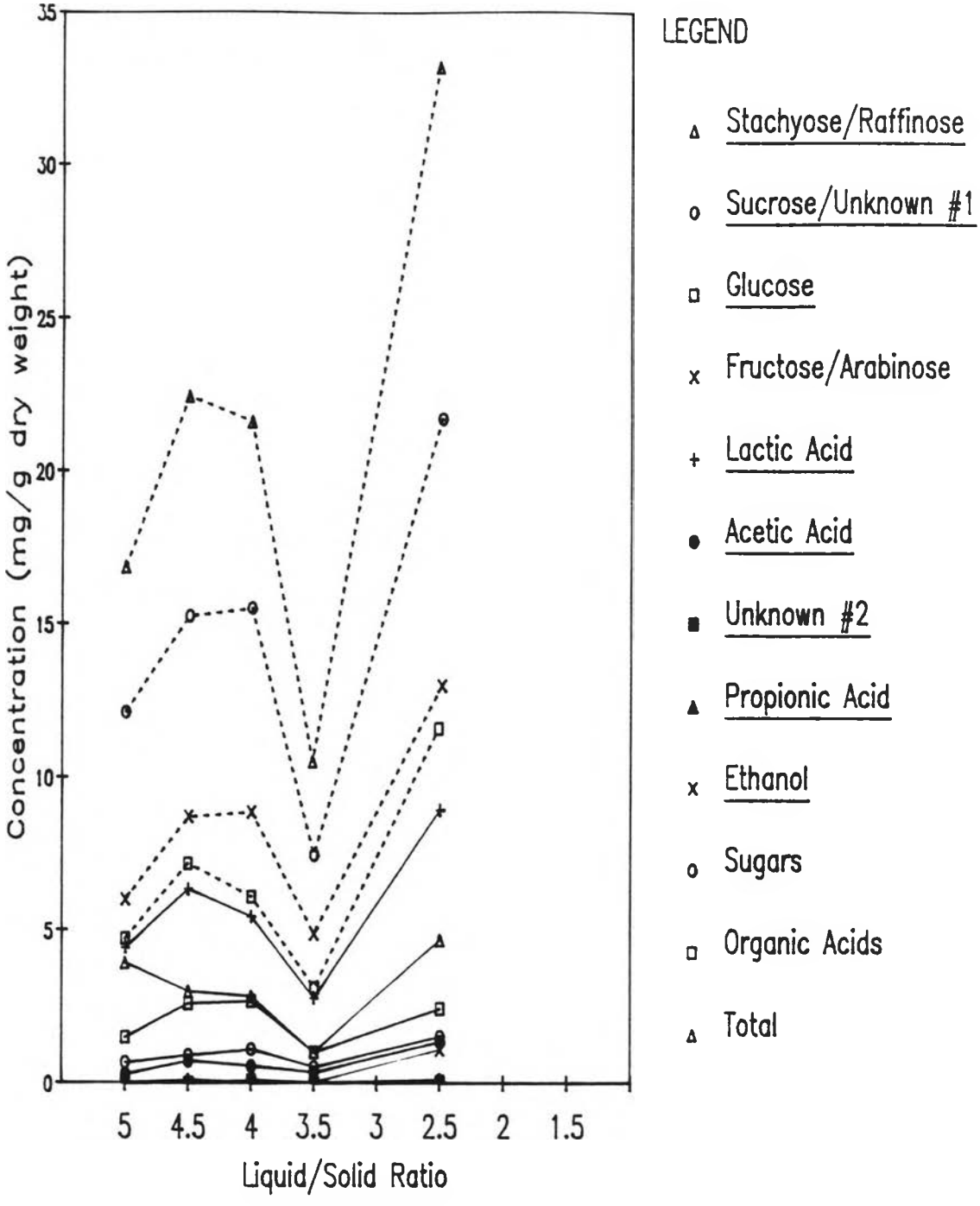


Figure 16. Pulp component concentrations at 50°C.

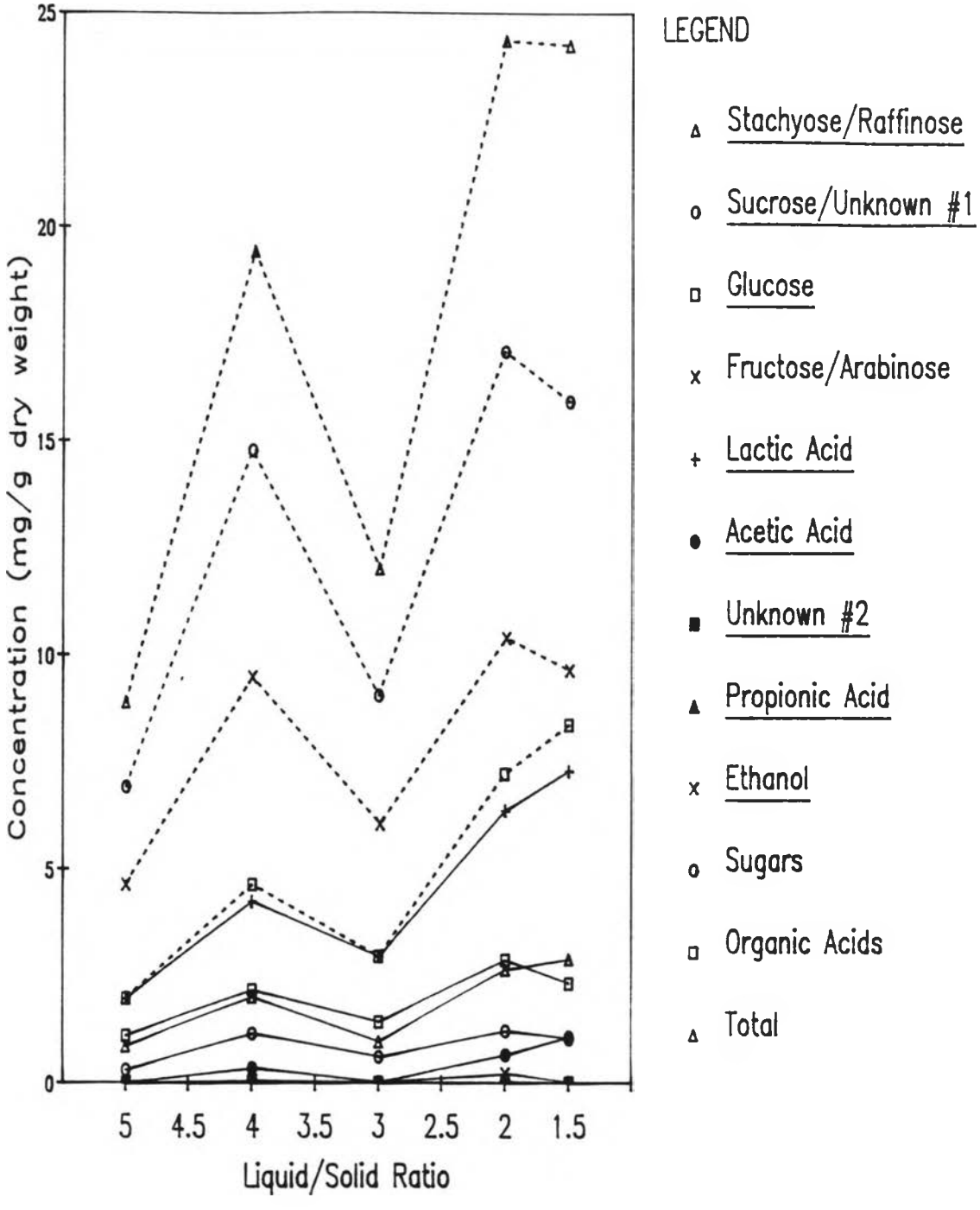


Figure 17. Pulp component concentrations at 70°C.

each new day. The other results, which were exceptions to the rule, were the low points for the 70°C 3.0 L/S ratio run. The reason for these low values was unknown. Possible reasons could be HPLC error, the sample was damaged in the freezer, something happened during the pressing of the sample, or operational fluctuation during that run.

Figures 18 and 19 show the concentration of each component in the diffusion juice during the 50°C and 70°C runs, respectively. As the L/S ratio decreased the component concentrations in the juice increased. This was due to the dilution effect at increased L/S ratios. The two exceptions were the 50°C and 70°C 4.0 L/S ratio runs. The 50°C, 4.0 L/S ratio values were high probably due to analysis error. These values gave extraction efficiencies on juice greater than 100 percent and juice component flowrates greater than the ensilage component flowrates, both of which are impossibilities. This point will be clarified later. The 70°C 4 L/S ratio values appeared low. This was also probably due to analysis error.

An important point to be made from Figures 18 and 19, was that the sugar in the diffusion juice was not of a sufficiently high concentration (6% w/v) for direct use in fermentation. The density of the diffusion juice was approximately 1 g/ml. Therefore, the highest concentration of total soluble components in the diffusion juice obtained was around 53 g/L at the 1.5 L/S ratio in the 70°C study. The concentration of total sugars was approximately 3 percent (w/v) at the 1.5 L/S ratio. In order to get a higher sugar concentration in the diffusion juice, lower L/S ratios would be needed. For the diffuser used in these experiments, lower L/S ratios would be hard to obtain; 1.5/1 was the lowest that could be achieved without causing

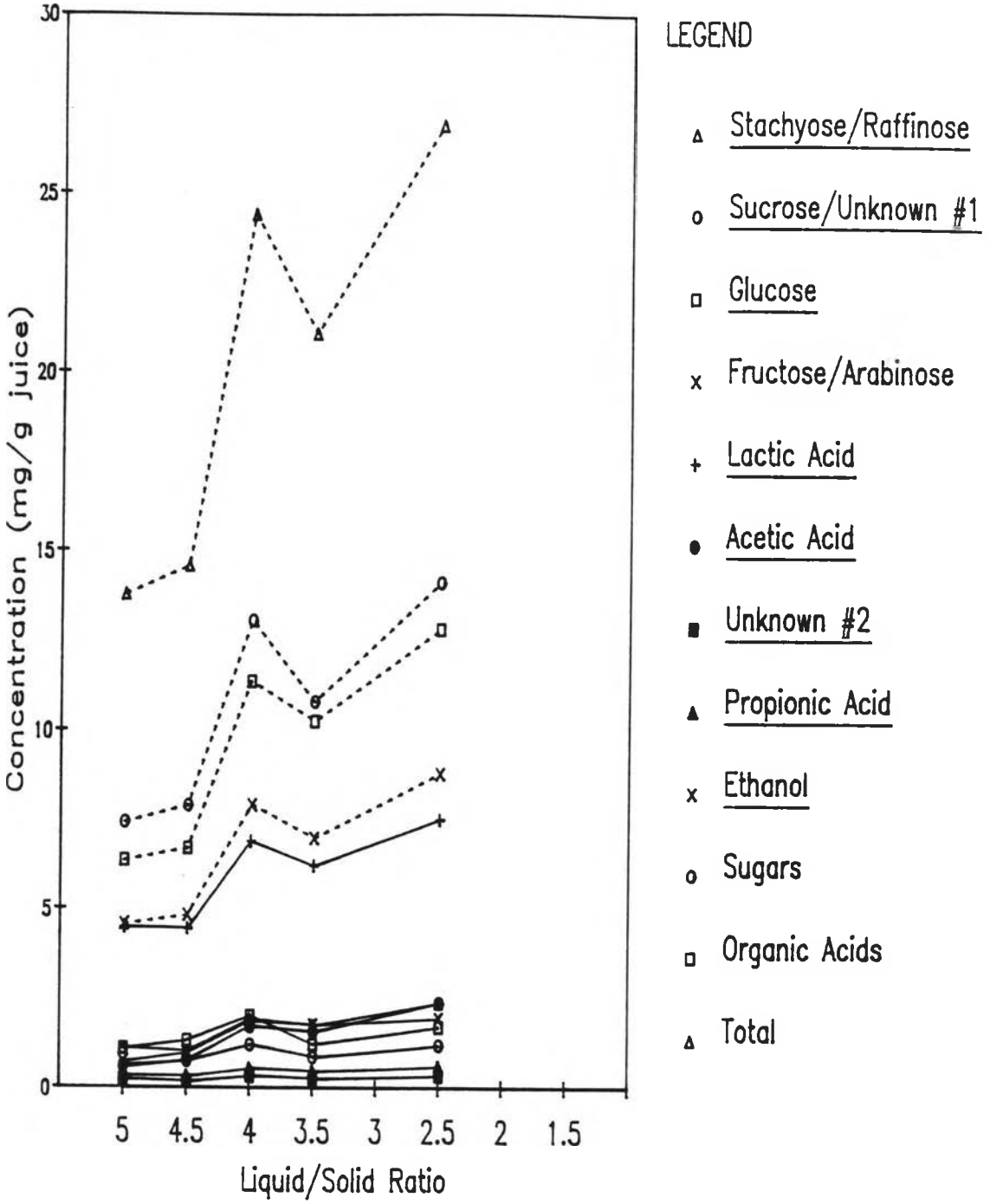


Figure 18. Juice component concentrations at 50°C.

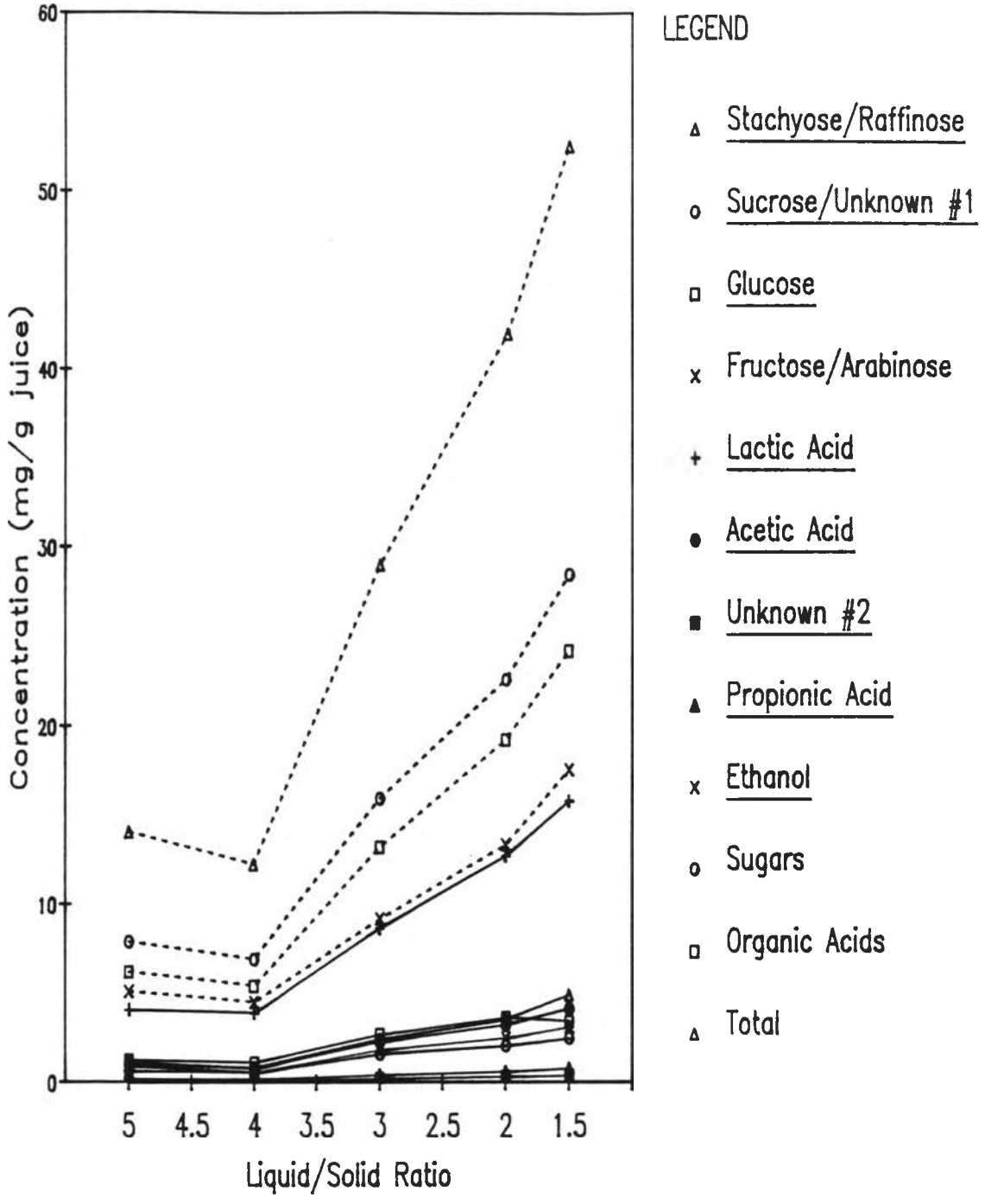


Figure 19. Juice component concentrations at 70°C.

constant clogging. Some of the chopped sorghum could fit through the perforated wiped plate, causing the diffusion juice tube to clog. Clogging did not occur that often at higher L/S ratios due to the higher mass flowrate of water pushing the bits of chopped sorghum that fit through the perforated wiped plate out of the diffusion juice tube. One possible way to solve the clogging problem would had been to put cheesecloth over the perforated plate to prevent the smaller pieces of sorghum from passing through the plate, or to use a smaller mesh perforated plate. A final possibility would be to use larger mesh chopped sorghum if the extraction would be as complete as with smaller mesh sizes. These problems would not be limiting using larger scale equipment.

Figure 20 shows the overall material balances for the diffuser; {Accumulation (g/min) = Water In (g/min) + Ensilage In (g wet sorghum/min) - Juice Out (g/min) - Pulp Out (g wet pulp/min)}. As mentioned earlier the L/S ratio was changed by keeping the mass feedrate of the sweet sorghum ensilage constant and varying the mass feedrate of the water. The figure shows this. The pulp mass flowrate (g wet pulp/min) for the 50^{o}C runs showed a decreasing trend with decreasing L/S ratio, while the 70^{o}C runs showed an increasing trend with decreasing L/S ratio except for the last two L/S ratios, 2.0 and 1.5. It was also interesting to note that the pulp mass flowrate for both temperatures was higher than the ensilage mass feedrate with the exception of the 50^{o}C 2.5 L/S run. This was due to the average increase in moisture content of the pulp relative to that of the ensilage. Pulp moistures were 84.65 $\pm$ 0.63 % and 85.43 $\pm$ 0.49 % for the 50^{o}C and 70^{o}C runs respectively, and that of the ensilage

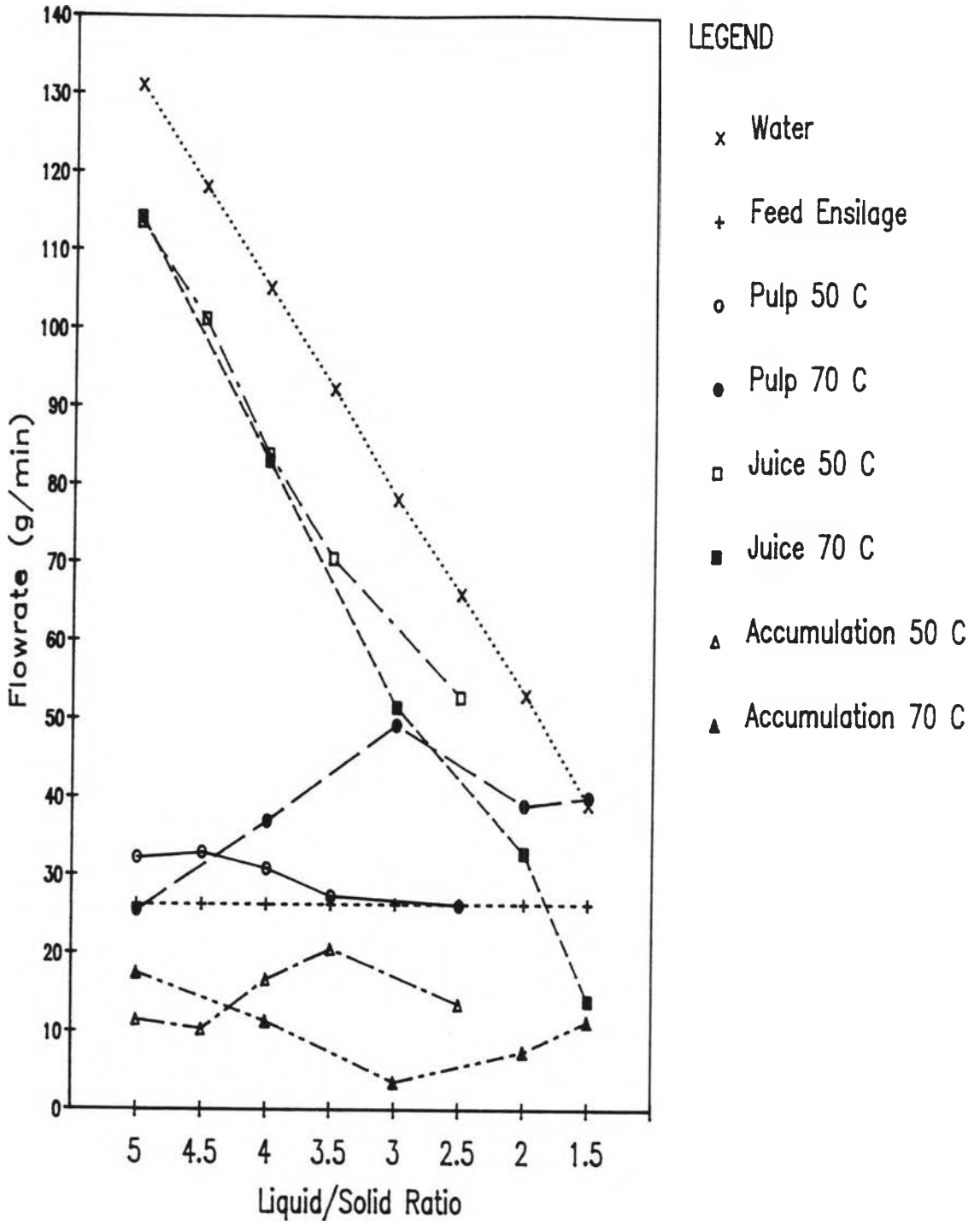


Figure 20. Diffuser overall material balances.

was 74.25 $\pm$ 2.05 %. One would expect as the L/S decreased, the moisture content of the pulp would decrease because there was less water to absorb, which would decrease the mass flowrates of the pulp with decreasing L/S ratios. Even though the 50^{o}C run showed this trend it was not due to the moisture content decreasing with decreasing L/S ratio; the moisture content varied widely in the samples taken from the studies at 50^{o}C between the 5.0 L/S ratio and the 2.5 L/S ratio. Also for the 70^{o}C runs, the moisture content showed an overall decreasing trend with decreasing L/S ratio, which does not account for the increased pulp mass flowrate between the 5.0 and the 3.0 L/S ratios.

This unaccountable increase or decrease of the pulp mass flowrate with decreasing L/S ratio may explain why the accumulation terms vary with trends opposite of the corresponding pulp flowrates. For instance, when the pulp rate increased with decreasing L/S ratio, the accumulation term dropped with decreasing L/S ratio. The inconsistent accumulation terms and pulp mass flowrates may be explained by two observations. The first was that pulp would build up at the high end of the diffuser before it was pushed out by the wiping blade. This could allow for deviation when measuring the pulp mass flowrate. The second was that it would take some time before there was enough buildup of sorghum on the scroll before it would move up the diffuser. Data was not taken until this buildup occurred, but the buildup of sorghum on the scrolls could have continued well into a run. Part of the reason for changing the water and not the solid rate was due to the buildup of material on the scroll.

The juice flowrates decreased with decreasing L/S ratio in a fashion parallel to the decreasing inlet water flowrate. The juice flowrates were smaller than the inlet water flowrate because the pulp gained water content. There was no consistent trend for the high or low values of the juice flowrates. One would expect if the pulp moisture content was lower, the juice flowrate would be high. This was true of the 50°C L/S = 2.5 run in which the moisture content of the pulp was lower than normal and the corresponding juice flowrate closer to the water flowrate. This however was not the case for the 70°C L/S = 2.0 run. In this case, the moisture content of the pulp was abnormally high, but so was the juice flowrate.

Referring back to Figures 16 and 17, the 70°C runs had lower total pulp component concentrations than did the 50°C runs. This would imply that the conditions of diffusion at the 70°C temperature were superior for extracting the sweet sorghum. However, this observation could be deceiving since the ensilage for the 50°C runs (FE 8/20 and 8/21) had slightly higher soluble component concentrations than did the ensilage for the 70°C runs (FE 8/22 and 8/23) as seen in Table 3. In other words the 70°C temperature had less to extract and therefore should have lower component concentrations in the pulp.

Therefore, a better way to compare the effects of temperature and L/S ratio was to define two extraction efficiencies, one based on pulp, the other on juice. The pulp extraction efficiency was defined as the fraction of a component removed from the ensilage.

$$\text{Pulp Extraction Efficiency} = \frac{\text{mg component in ensilage} - \text{mg component in pulp}}{\text{mg component in ensilage}} \times 100 \quad (3)$$

The juice extraction efficiency was defined as the fraction of a component removed from the ensilage that wound up in the juice; the amount of a component in the juice divided by that in the ensilage.

$$\text{Juice Extraction Efficiency} = \frac{\text{mg component in juice}}{\text{mg component in ensilage}} \times 100 \qquad (4)$$

Since the mass flowrates of each component in the ensilage, pulp, and juice, were used to determine the extraction efficiencies based on pulp and juice, these parameters and the accumulation term from the mass balance on each component were plotted along with the extraction efficiency for the 50°C and 70°C studies in Figures 21 A to L and 22 A to L, respectively.

For both the 50°C and 70°C runs there were two values for the mass flowrate of the ensilage, one for the first day of runs and one for the second day of runs. For the 50°C temperature the first day of runs were at the 5.0, 4.5, and 4.0 L/S ratios, and the second day of runs were at the 3.5 and 2.5 L/S ratios. For the 70°C temperature the first day of runs were at the 5.0, 4.0, 3.0, and 2.0 L/S ratios, and the second day at the 1.5 L/S ratio. The higher or lower mass flowrate of a component on the second day corresponds with the higher or lower value of the concentration of that component in the ensilage on that day (table 3), since the wet sorghum feed rate was kept constant at 26.3 g/min throughout the experiment.

The pulp component flowrates were calculated by multiplying the wet pulp mass flow rate by the percent dry matter and by the component concentration. However, the component concentration played a bigger factor. The shape of the pulp component flowrate curves for both the 50°C and 70°C runs follows the shape of the pulp concentration

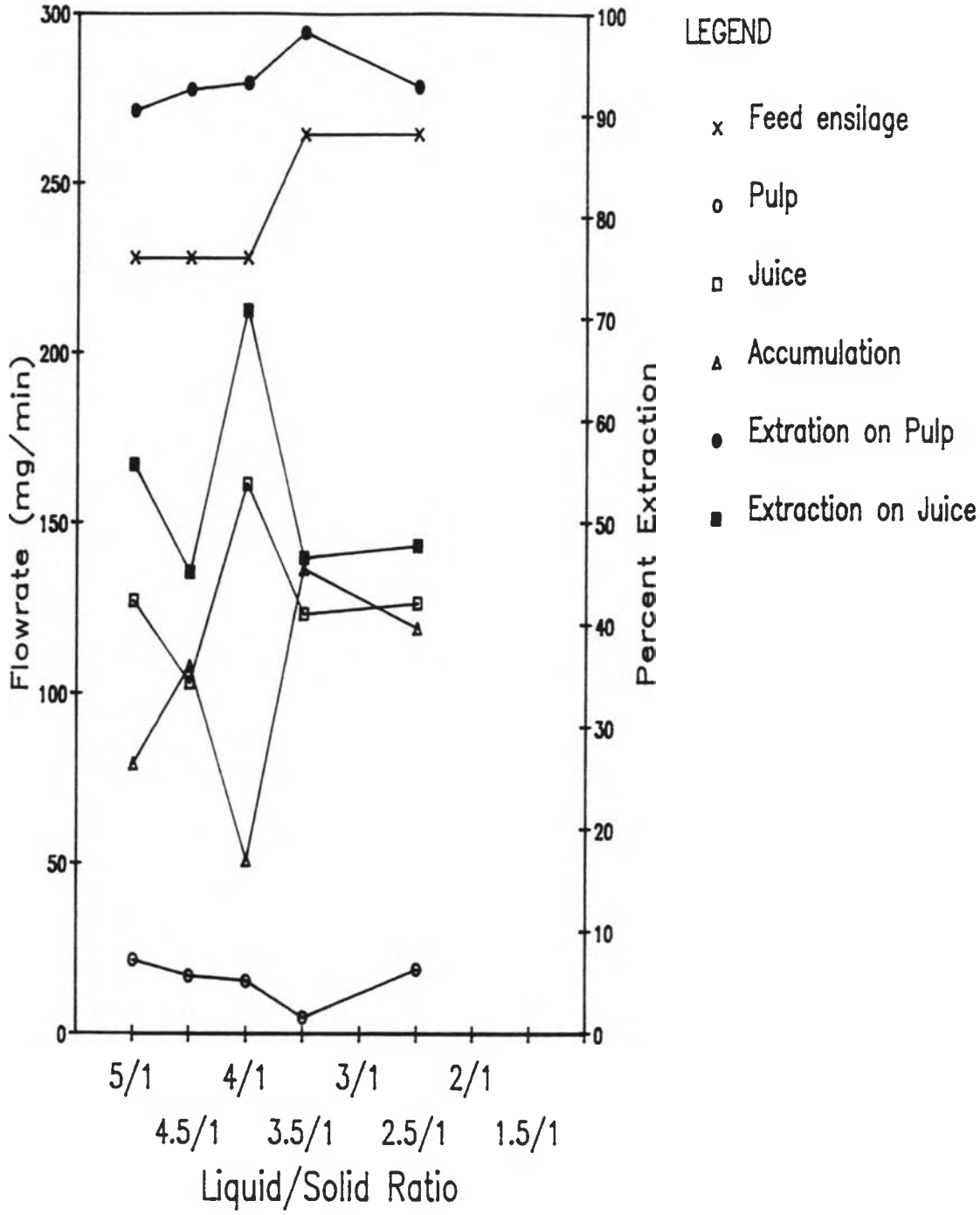


Figure 21A. Diffuser flowrates and extraction percentages for stachyose/raffinose at 50°C.

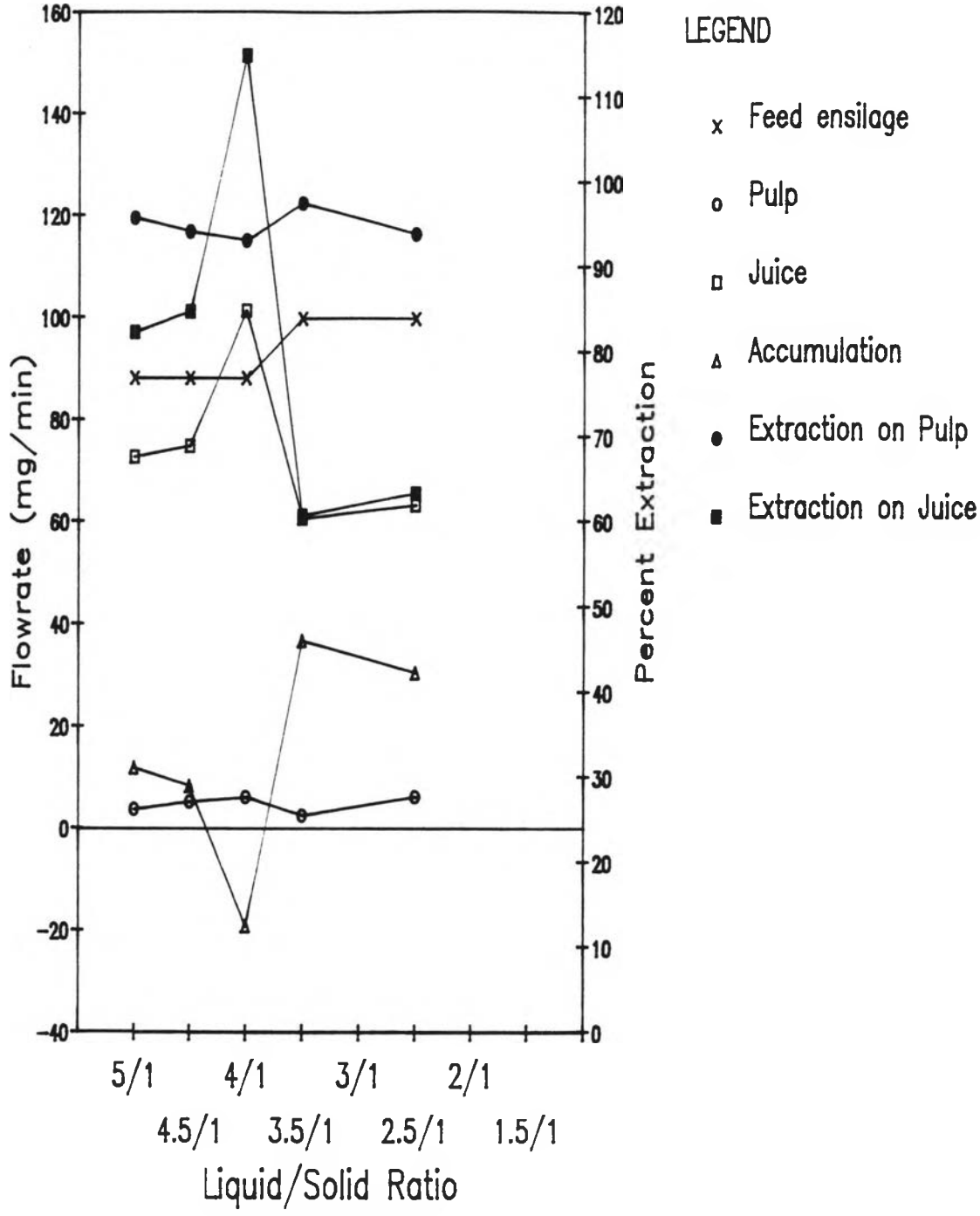


Figure 21B. Diffuser flowrates and extraction percentages for sucrose/unknown #1 at 50°C.

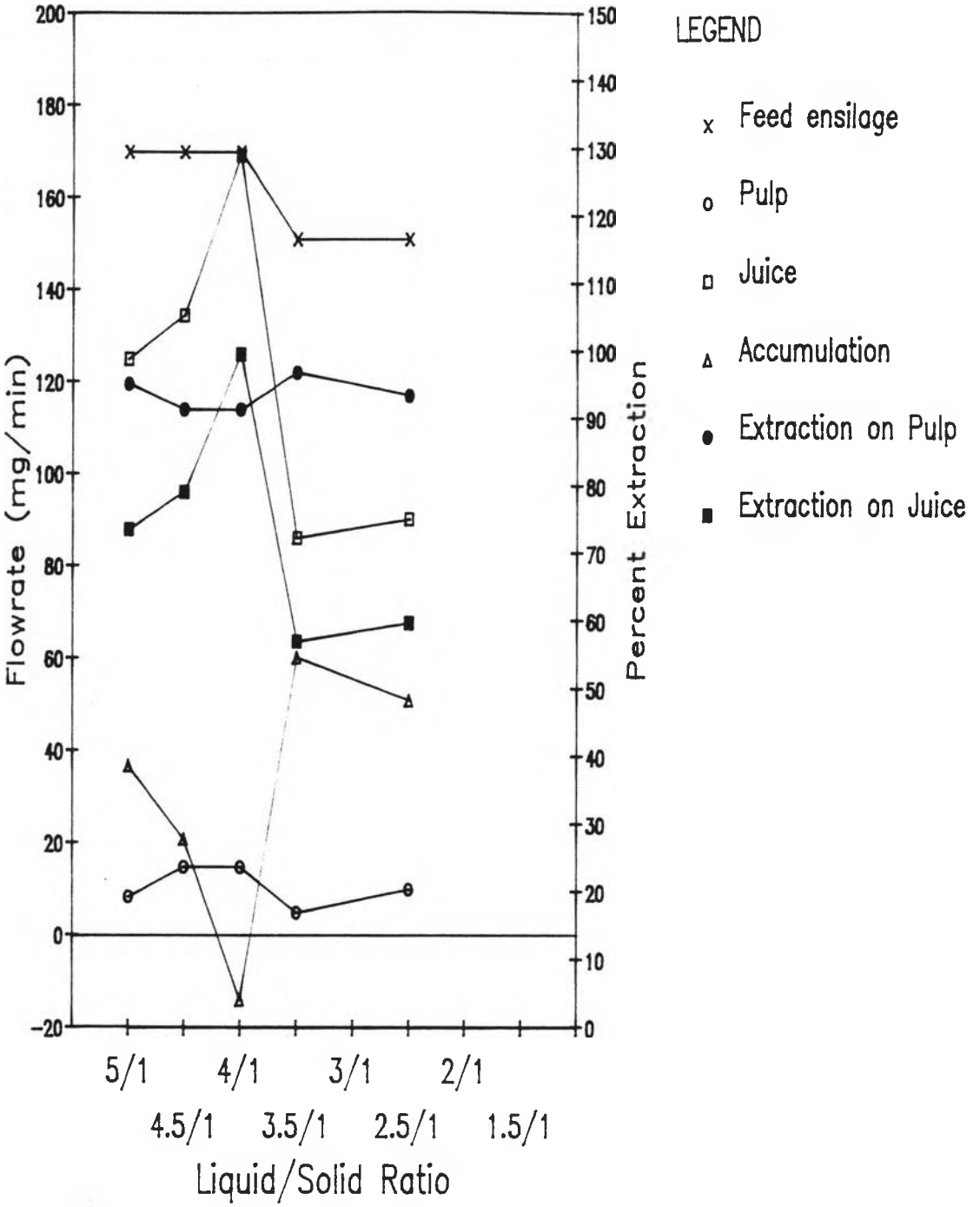


Figure 21C. Diffuser flowrates and extraction percentages for glucose at 50°C.

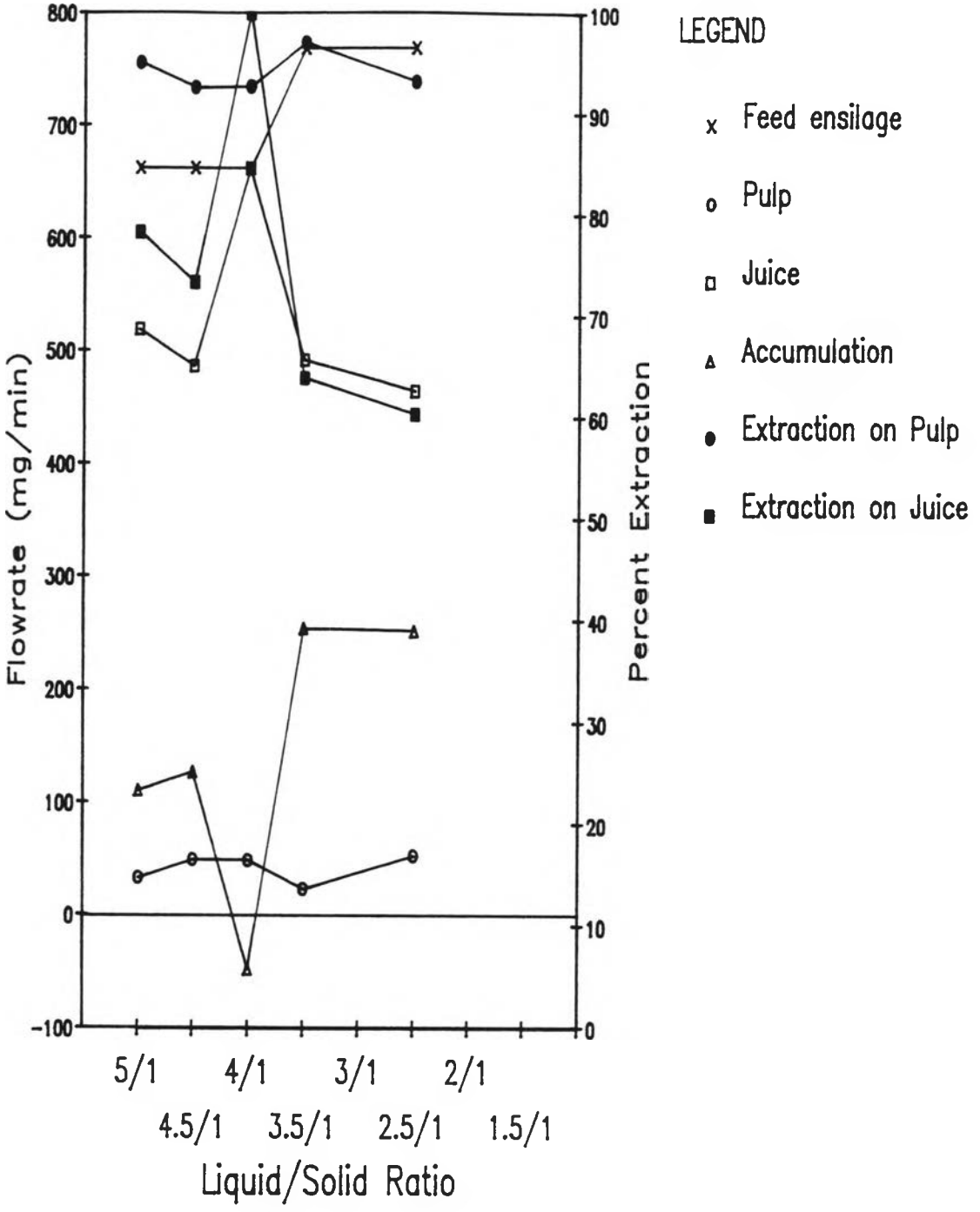


Figure 21D. Diffuser flowrates and extraction percentages for fructose/arabinose at 50°C.

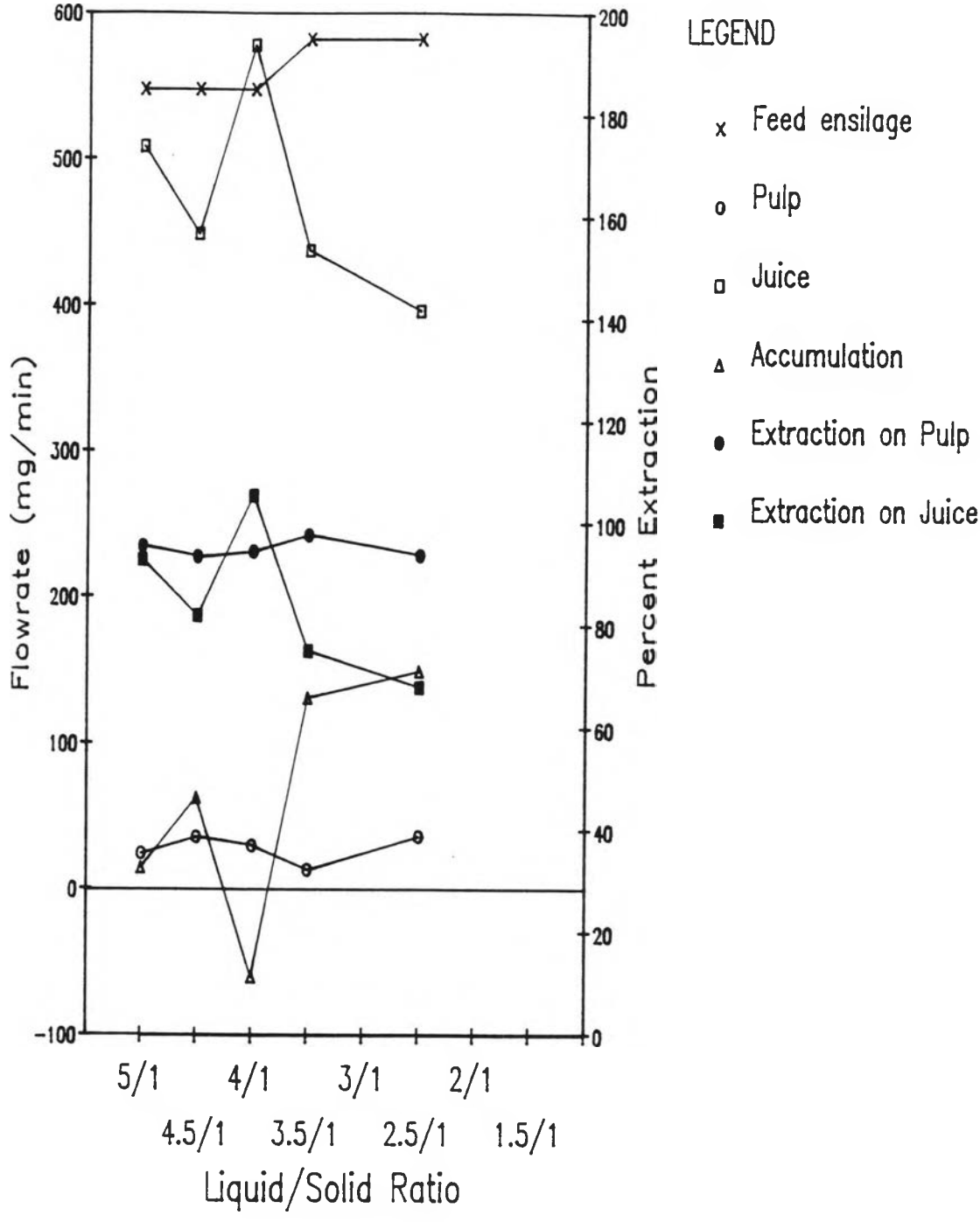


Figure 21E. Diffuser flowrates and extraction percentages for lactic acid at 50°C.

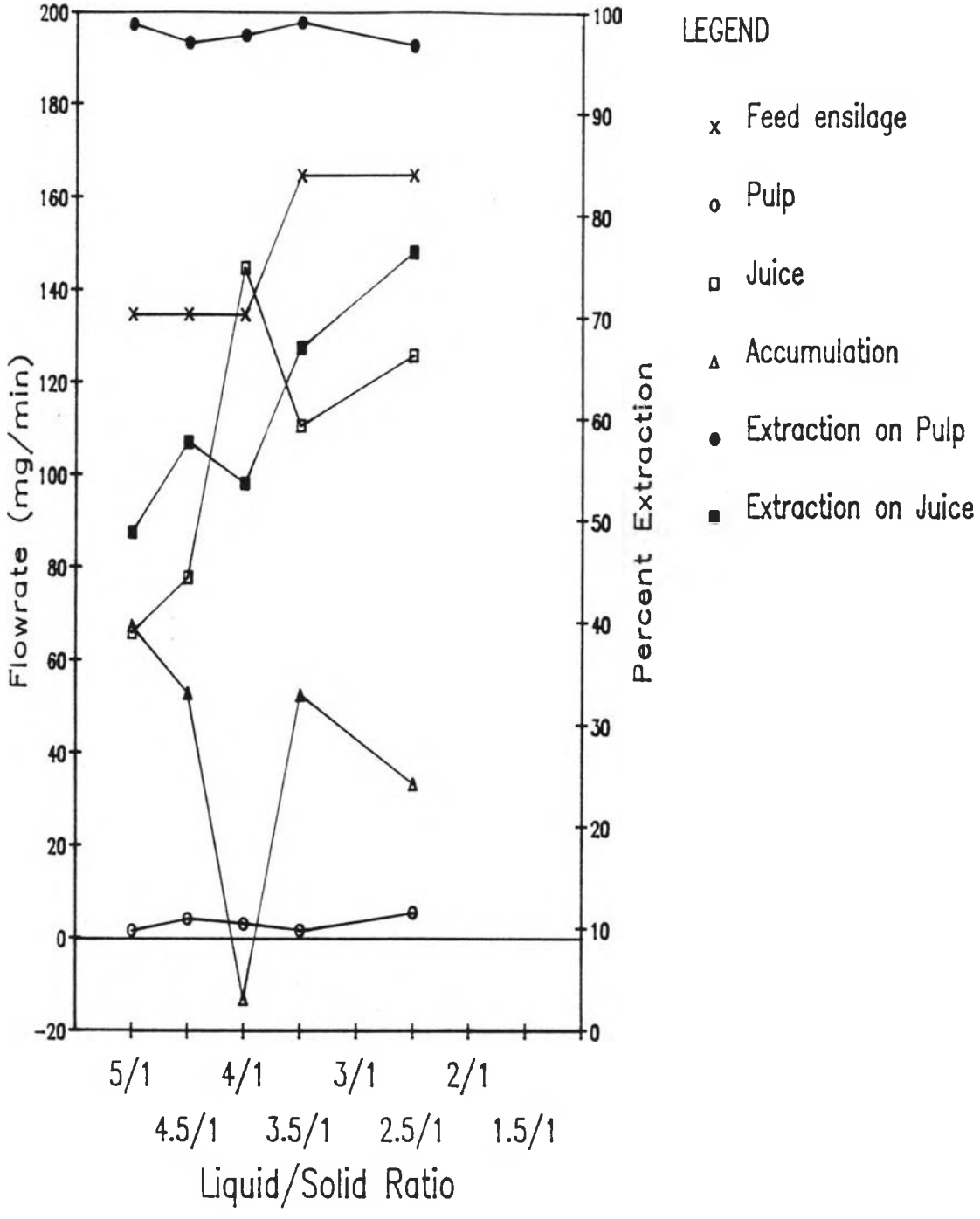


Figure 21F. Diffuser flowrates and extraction percentages for acetic acid at 50°C.

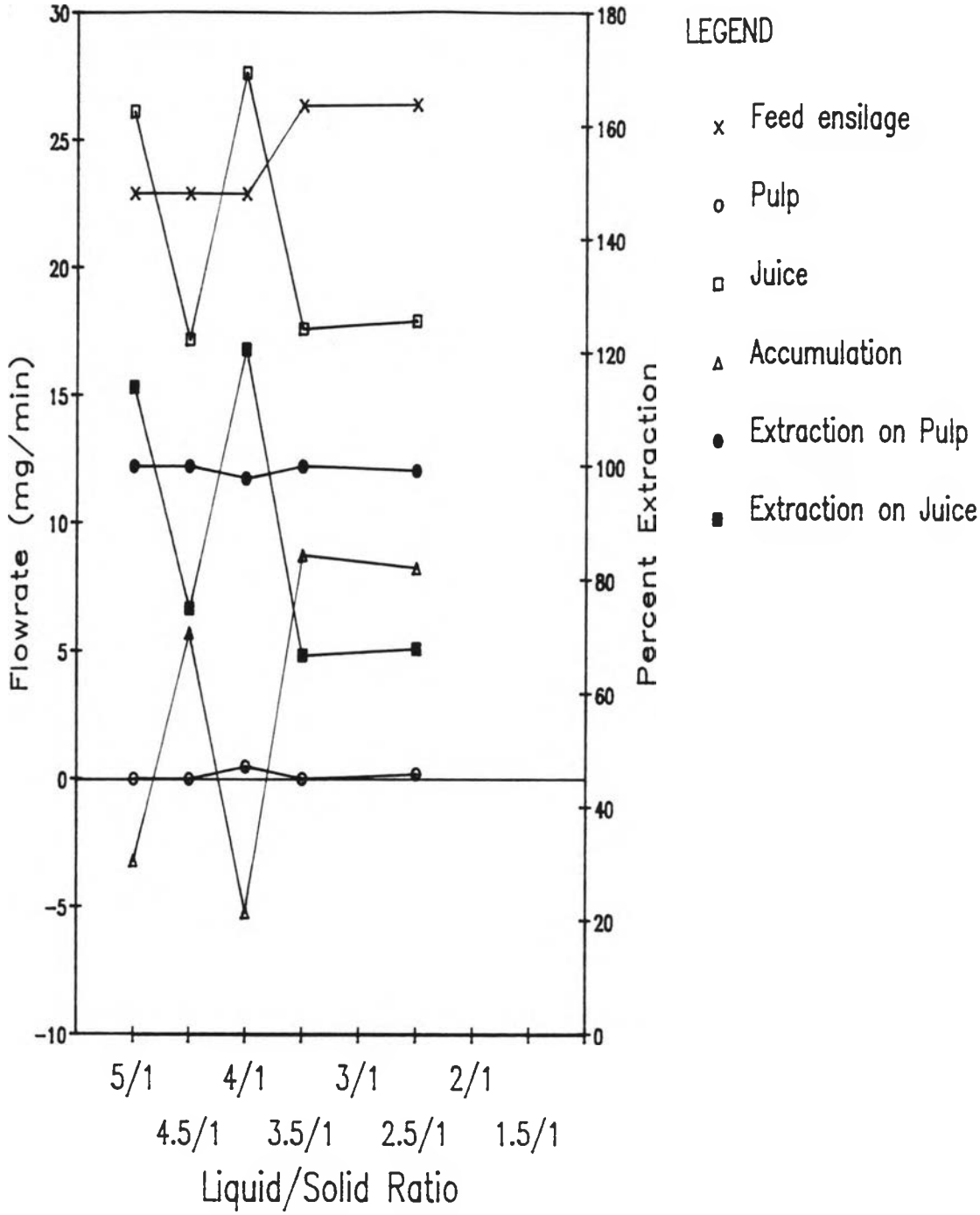


Figure 21G. Diffuser flowrates and extraction percentages for unknown #2 at 50°C.

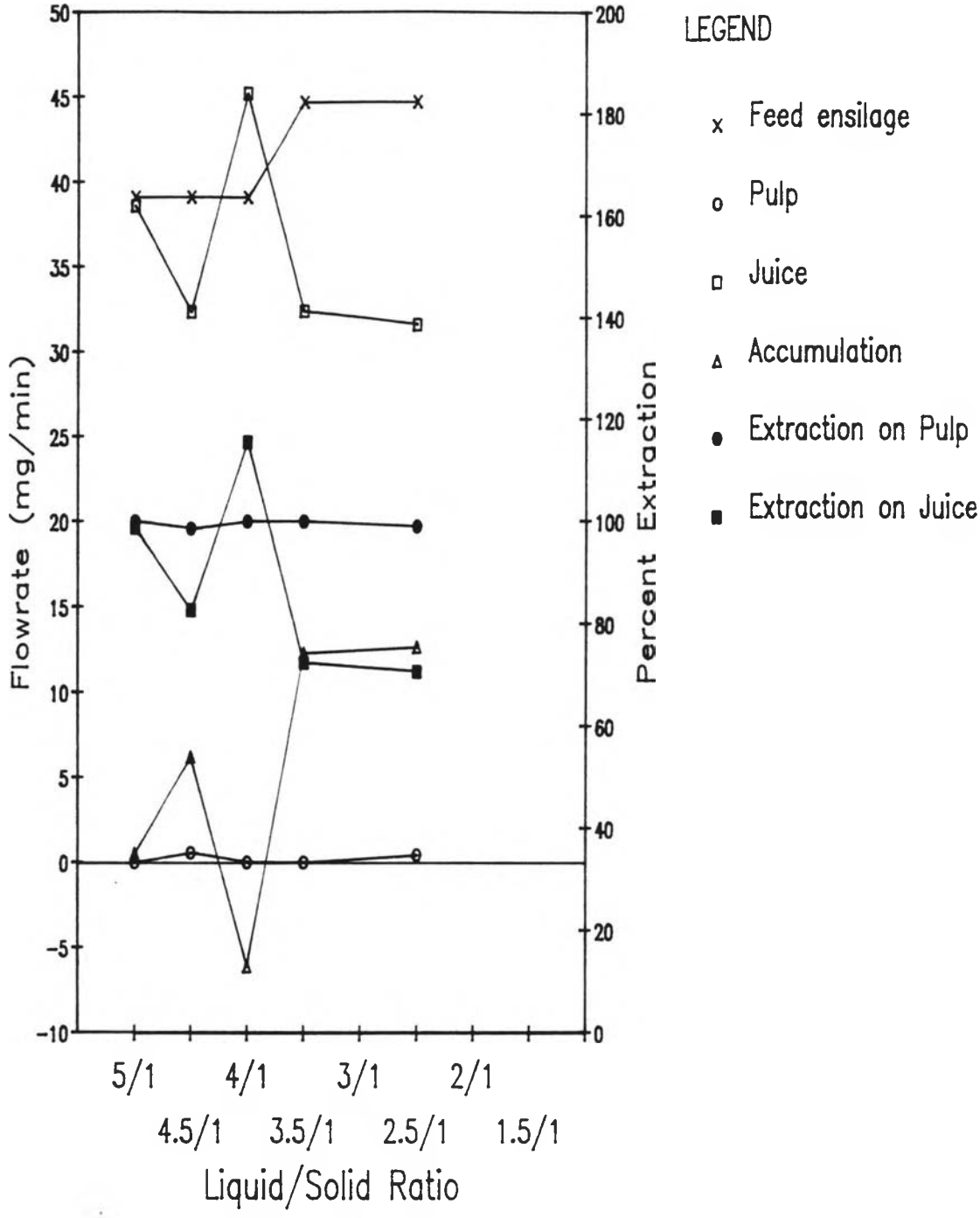


Figure 21H. Diffuser flowrates and extraction percentages for propionic acid at 50°C.

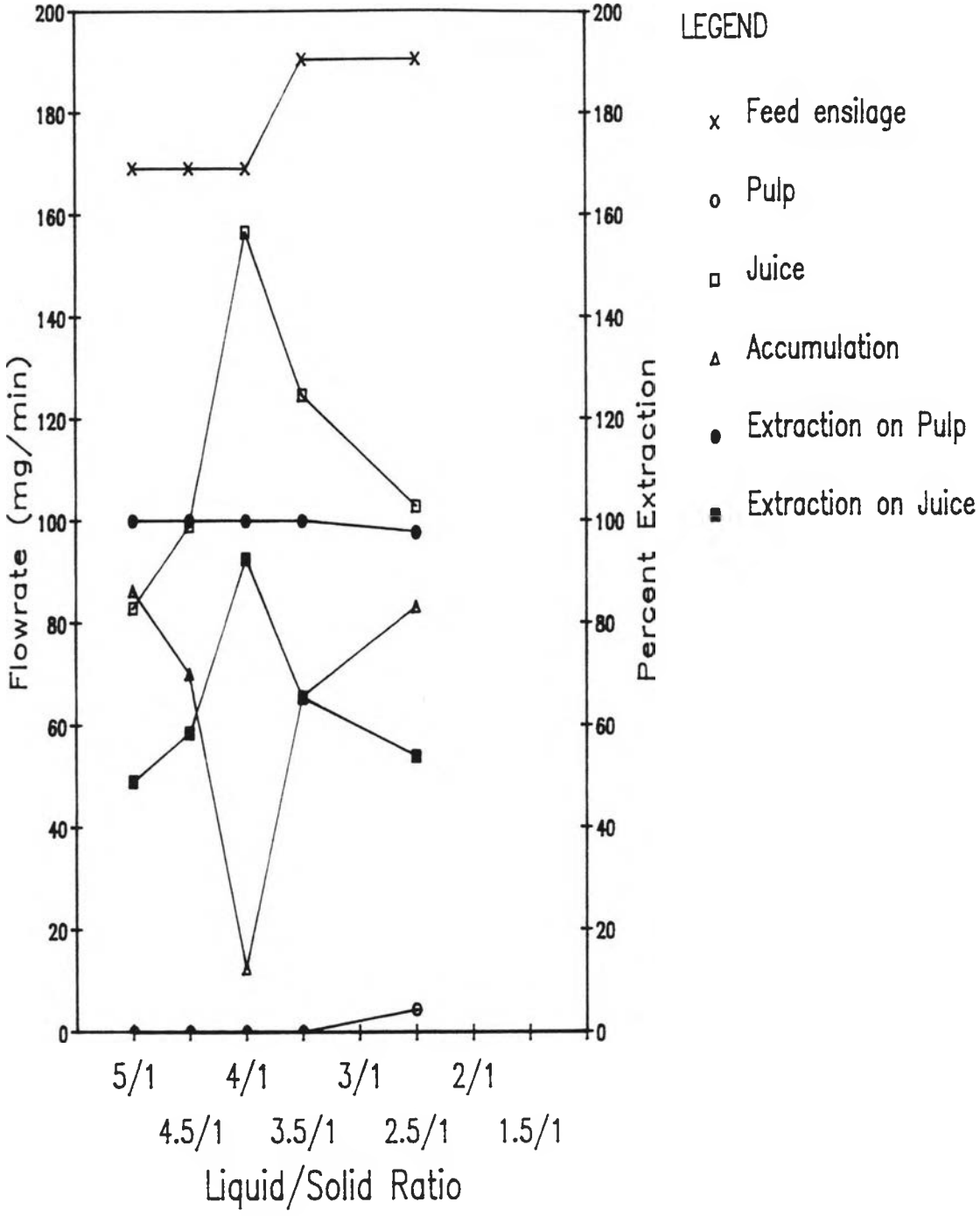


Figure 21I. Diffuser flowrates and extraction percentages for ethanol at 50°C.

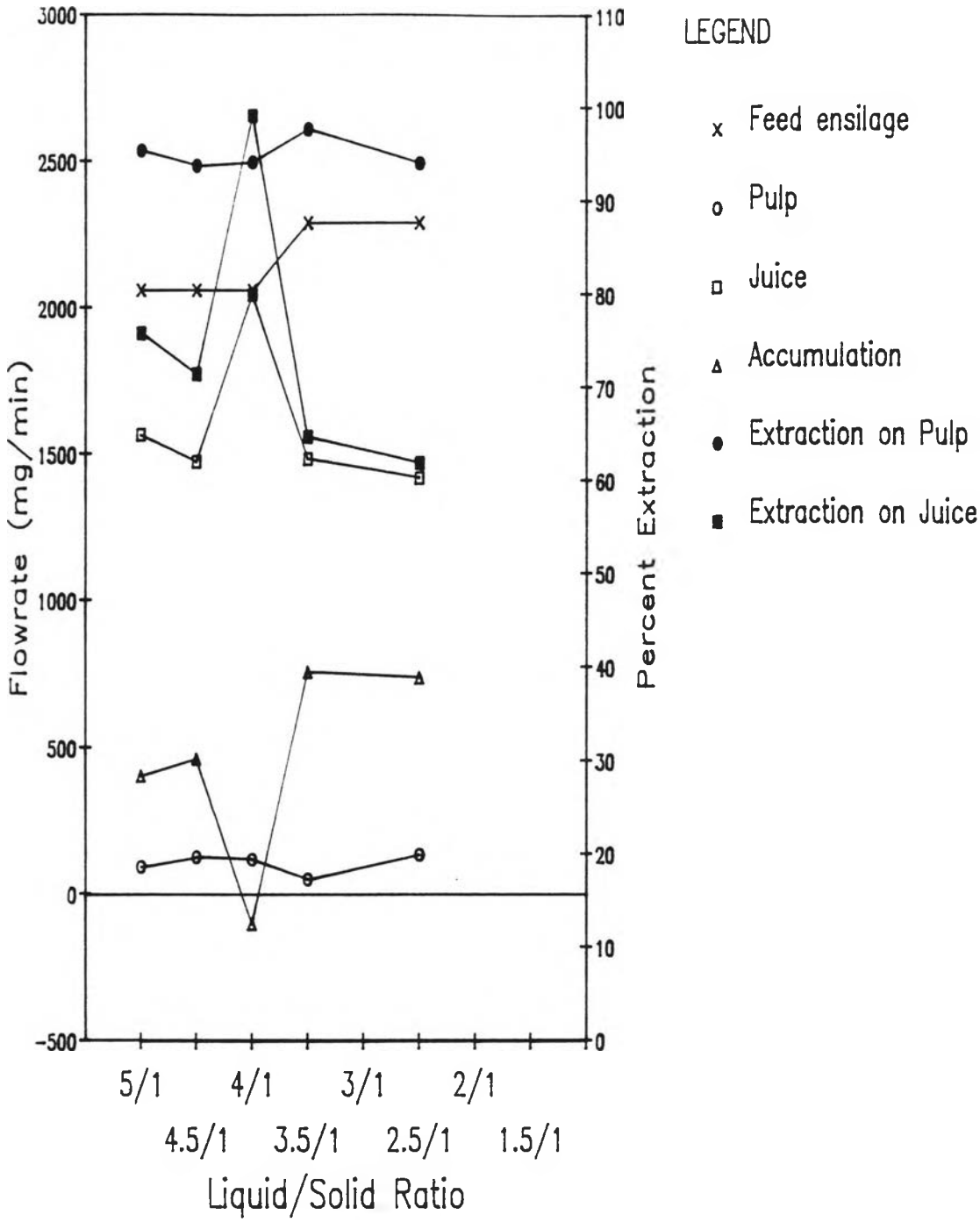


Figure 21J. Diffuser flowrates and extraction percentages for all components at 50°C.

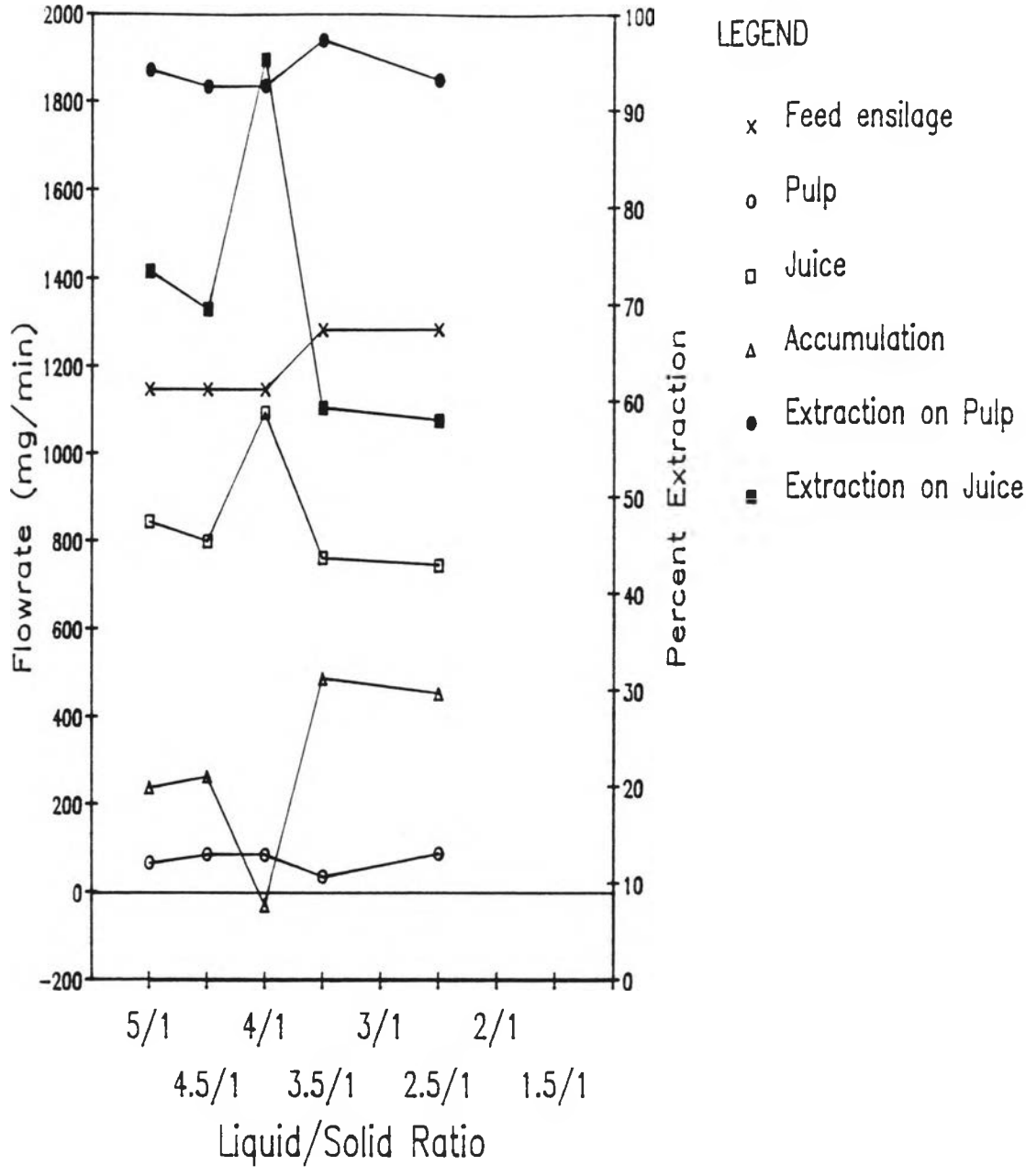


Figure 21K. Diffuser flowrates and extraction percentages for sugars at 50°C.

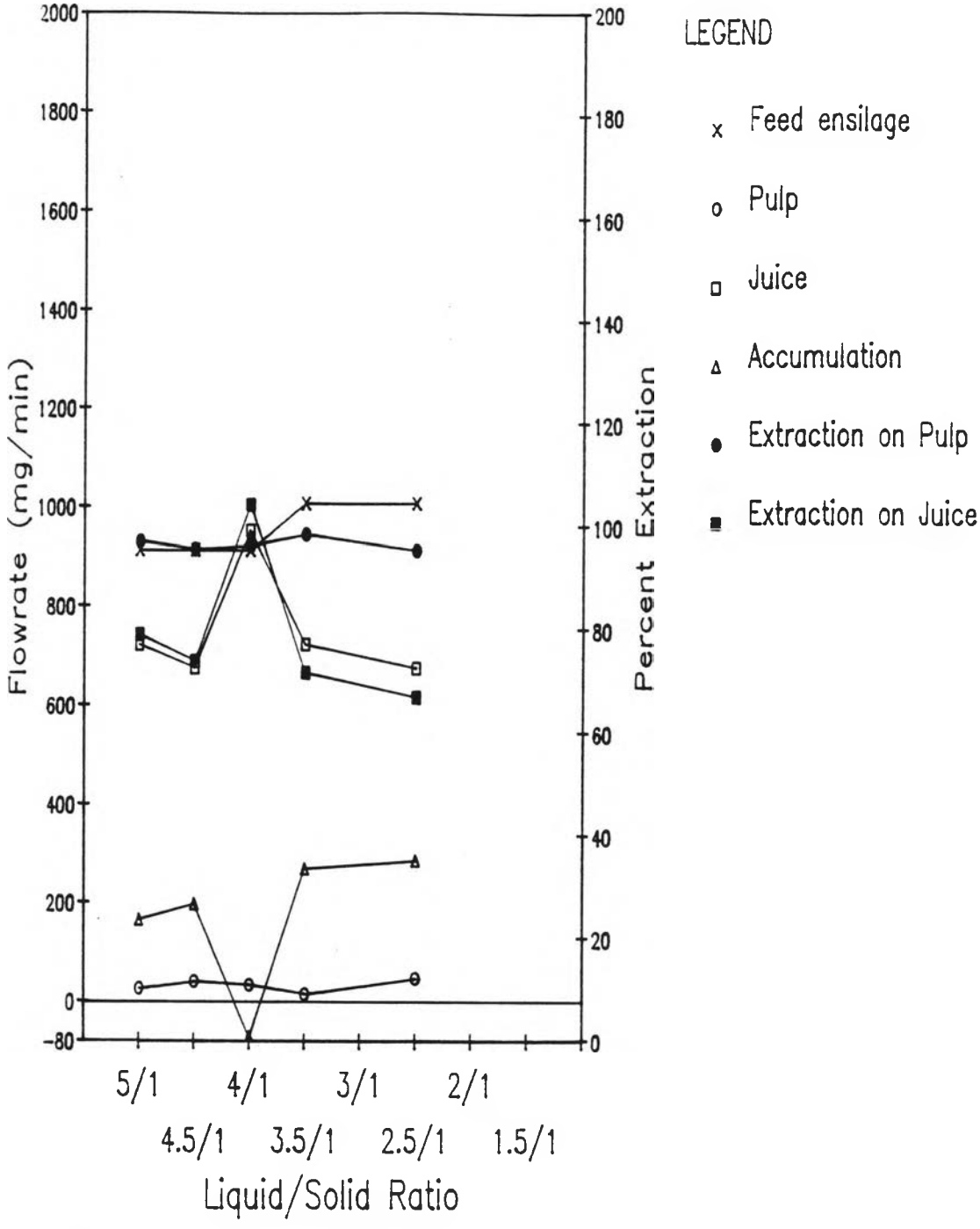


Figure 21L. Diffuser flowrates and extraction percentages for organic acids at 50°C.

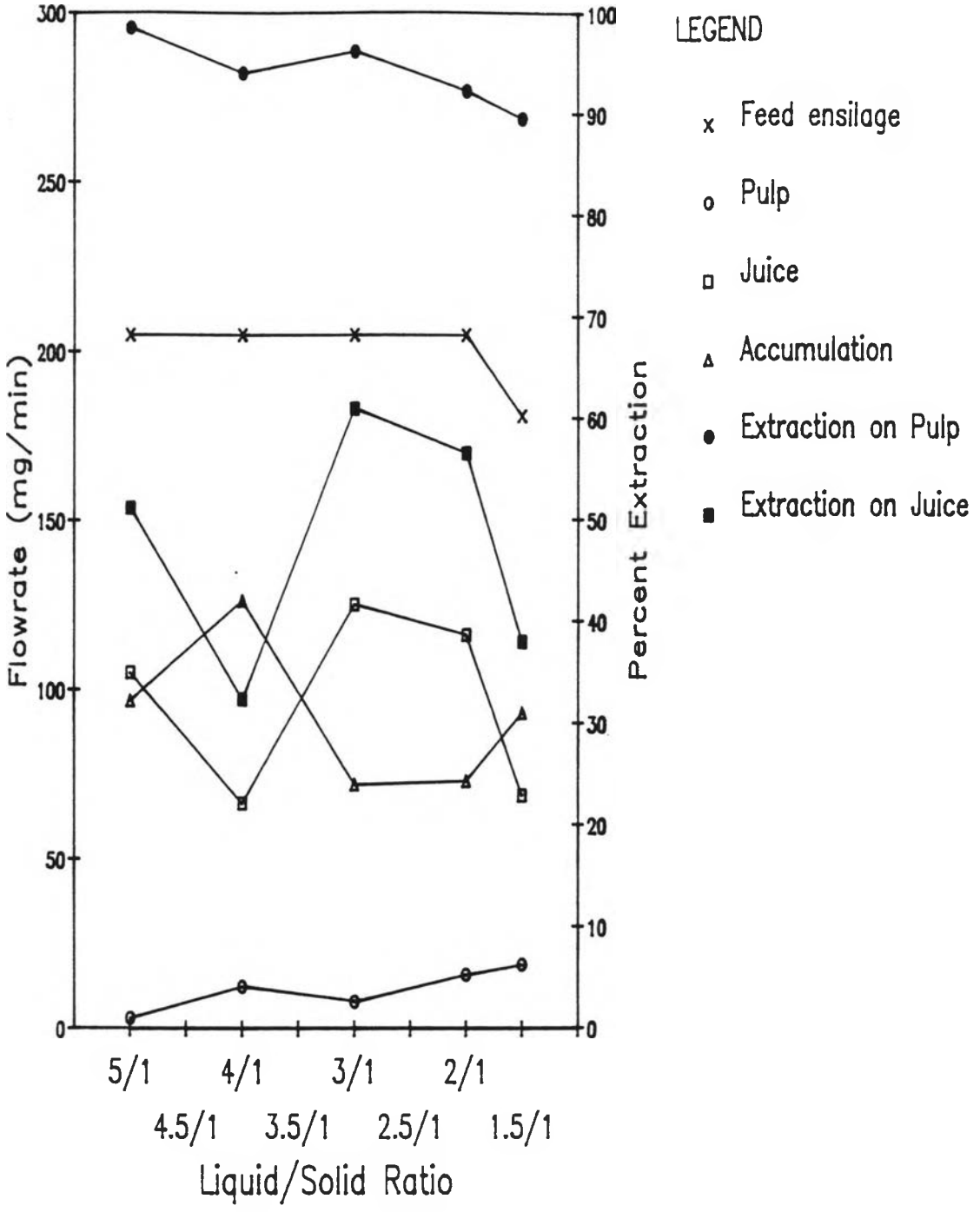


Figure 22A. Diffuser flowrates and extraction percentages for stachyose/raffinose at 70°C.

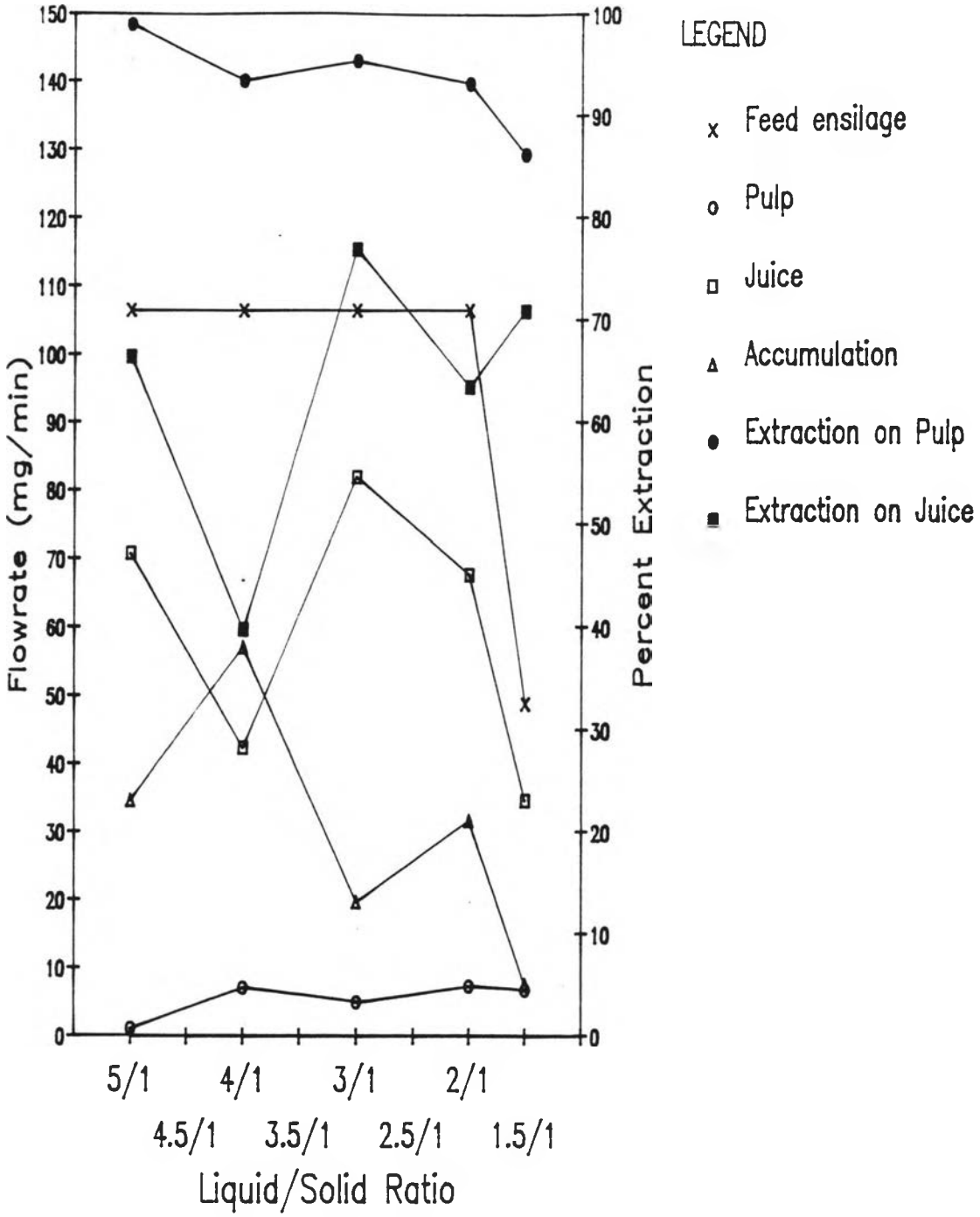


Figure 22B. Diffuser flowrates and extraction percentages for sucrose/unknown #1 at 70°C.

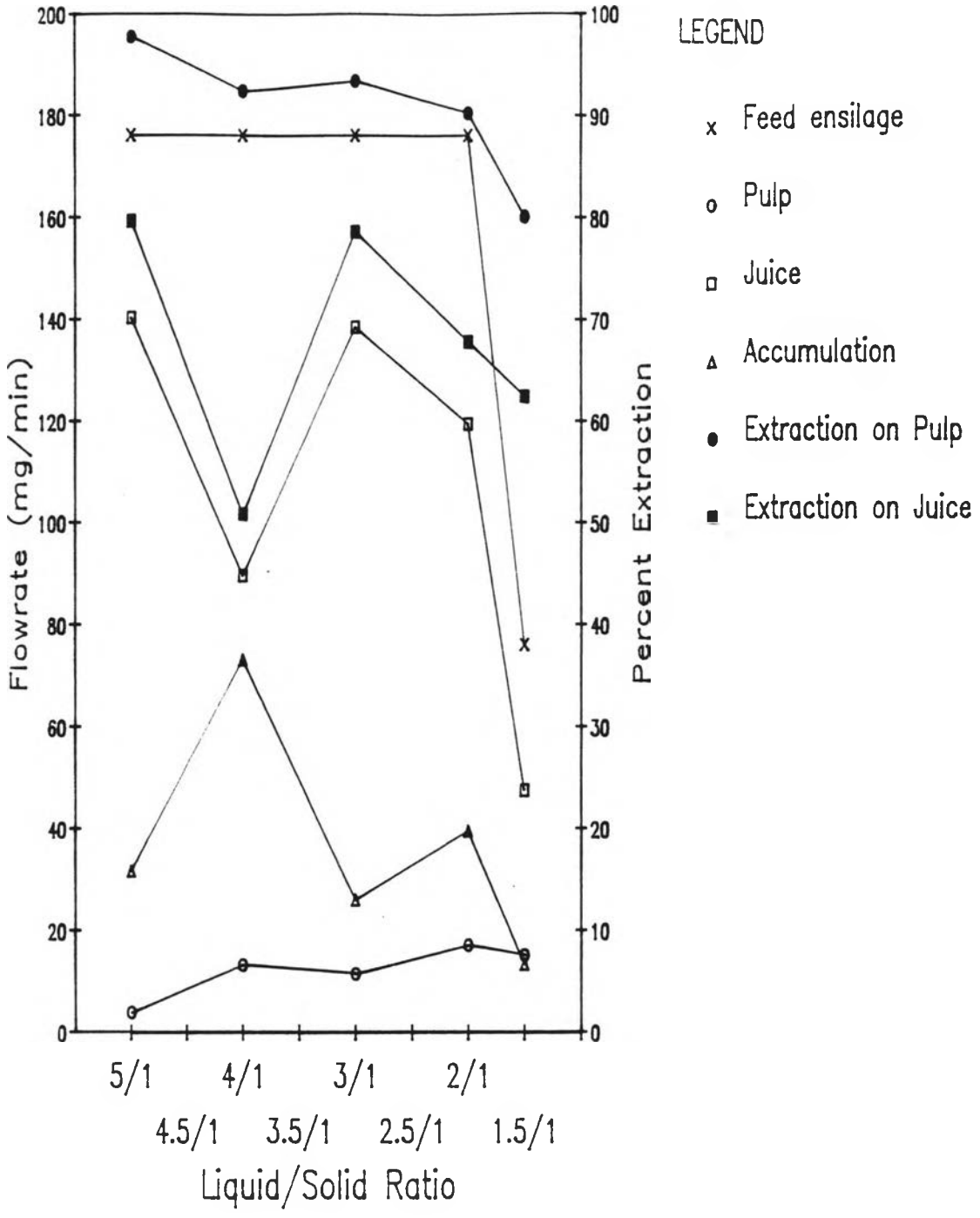


Figure 22C. Diffuser flowrates and extraction percentages for glucose at 70°C.

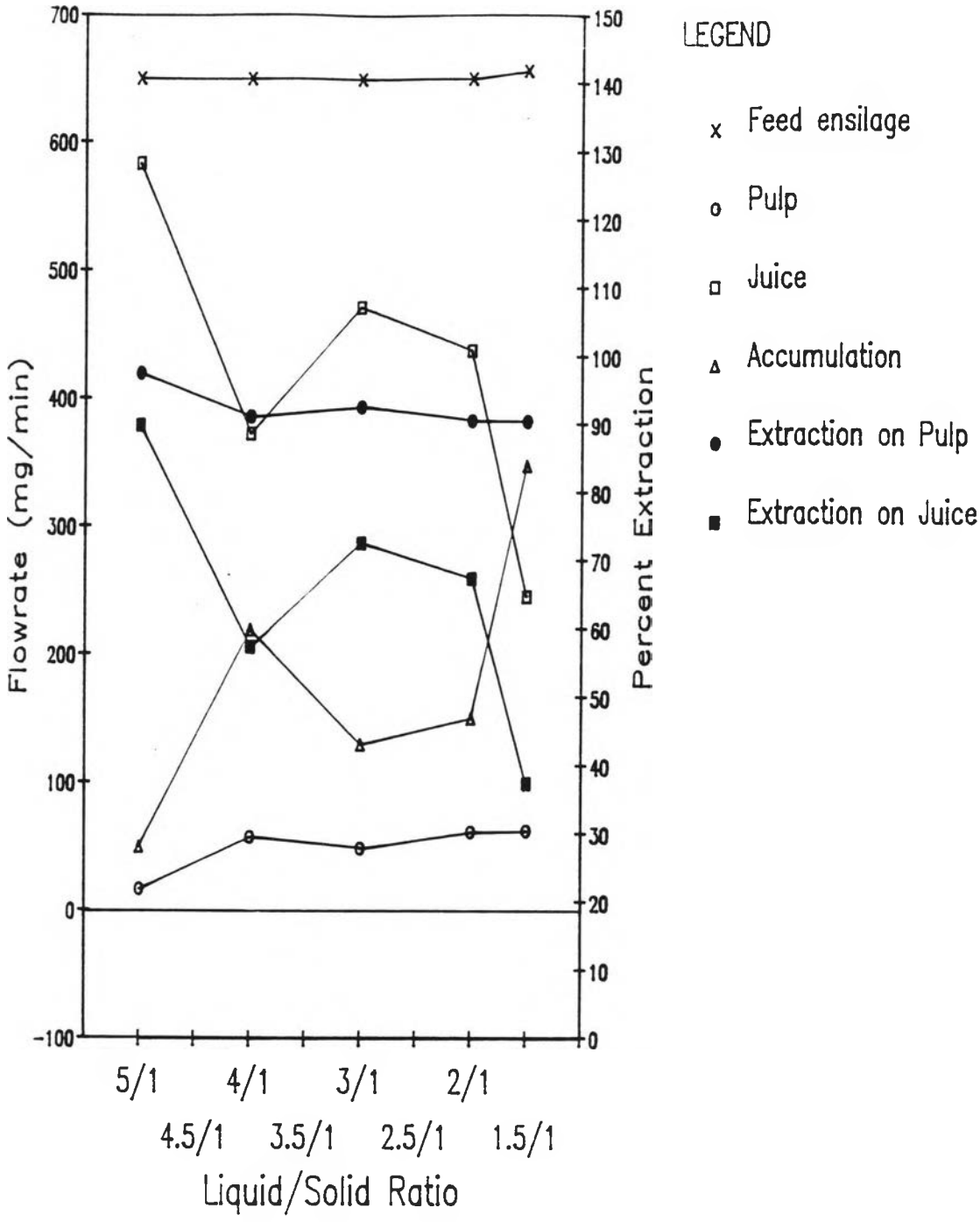


Figure 22D. Diffuser flowrates and extraction percentages for fructose/arabinose at 70°C.

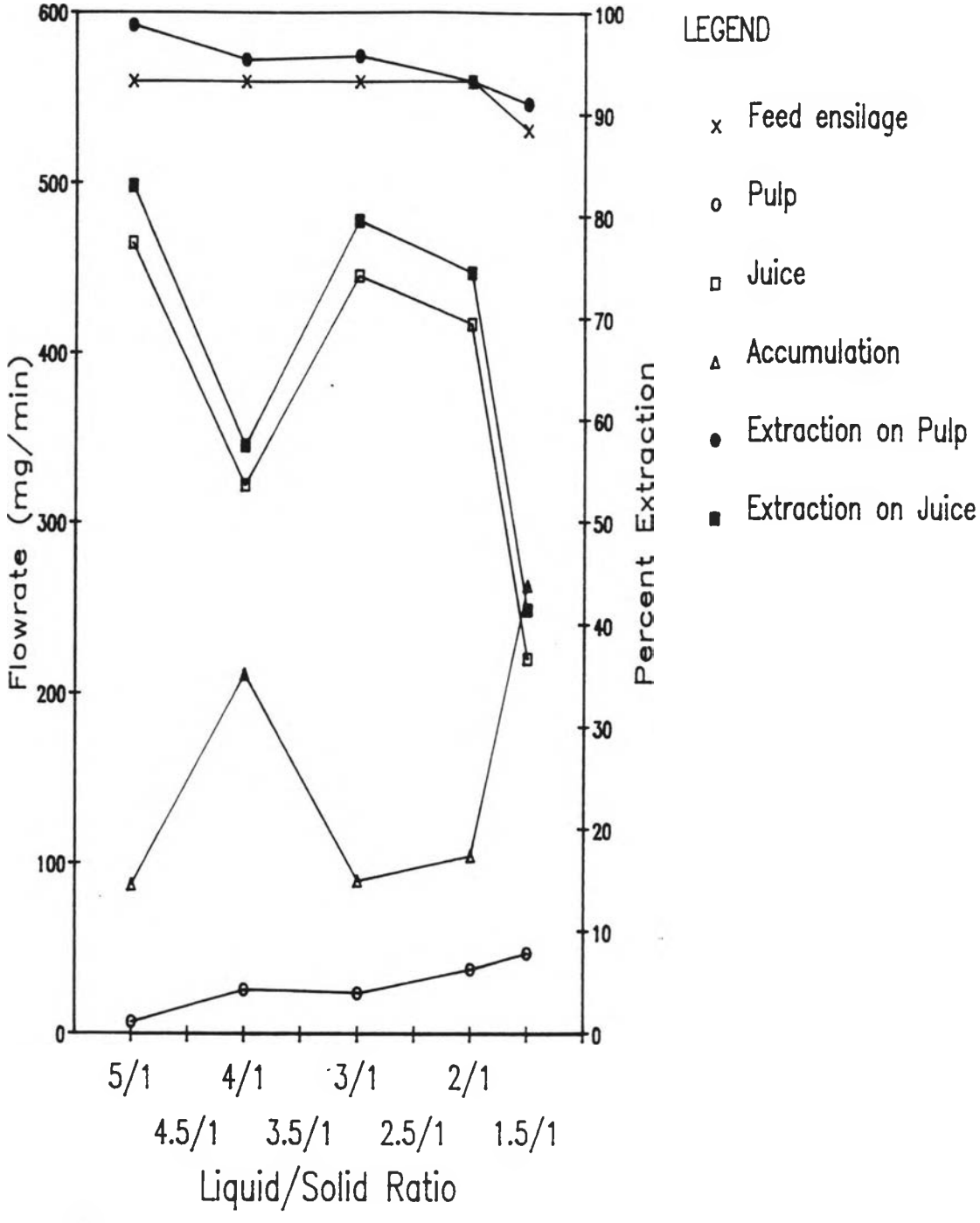


Figure 22E. Diffuser flowrates and extraction percentages for lactic acid at 70°C.

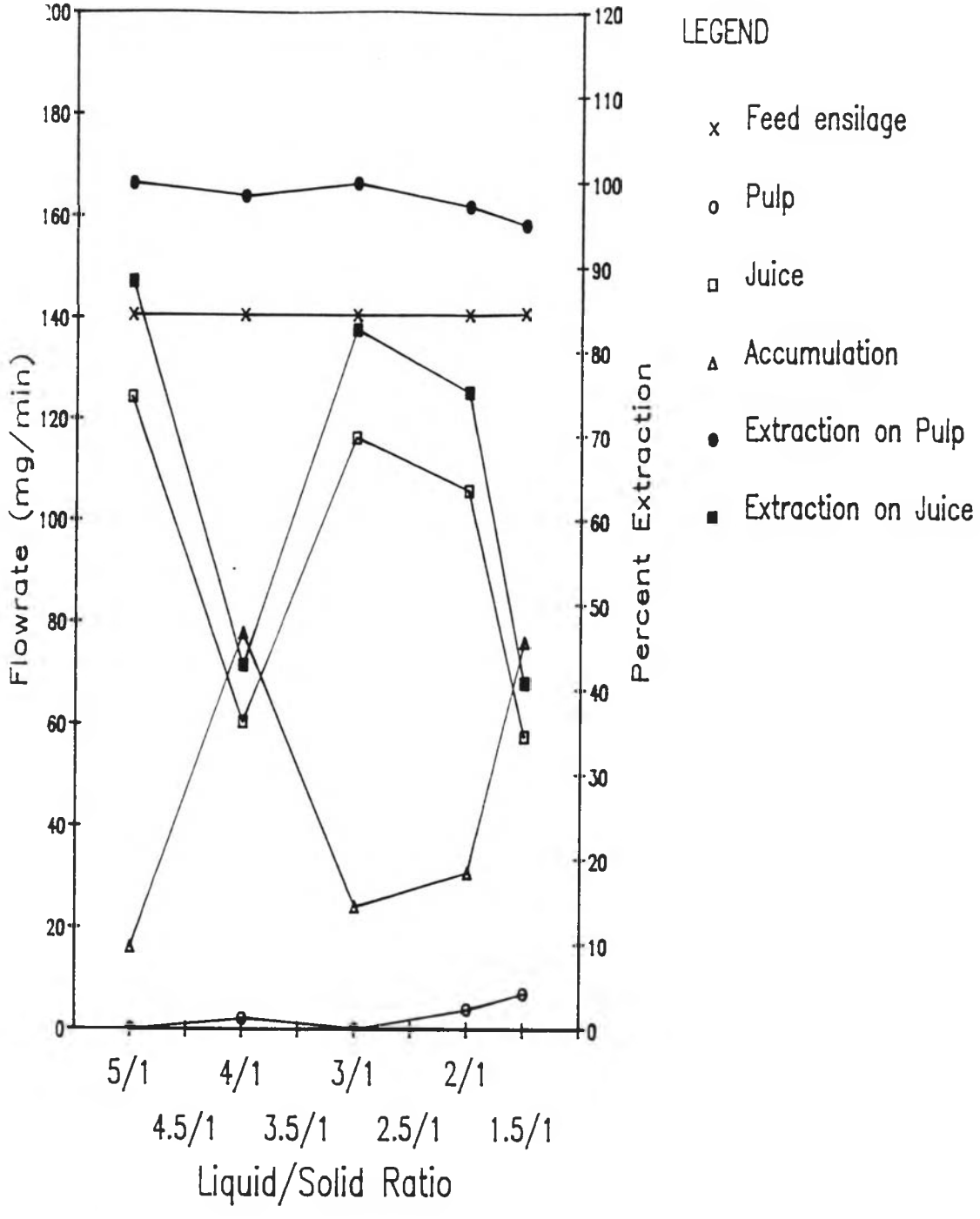


Figure 22F. Diffuser flowrates and extraction percentages for acetic acid at 70°C.

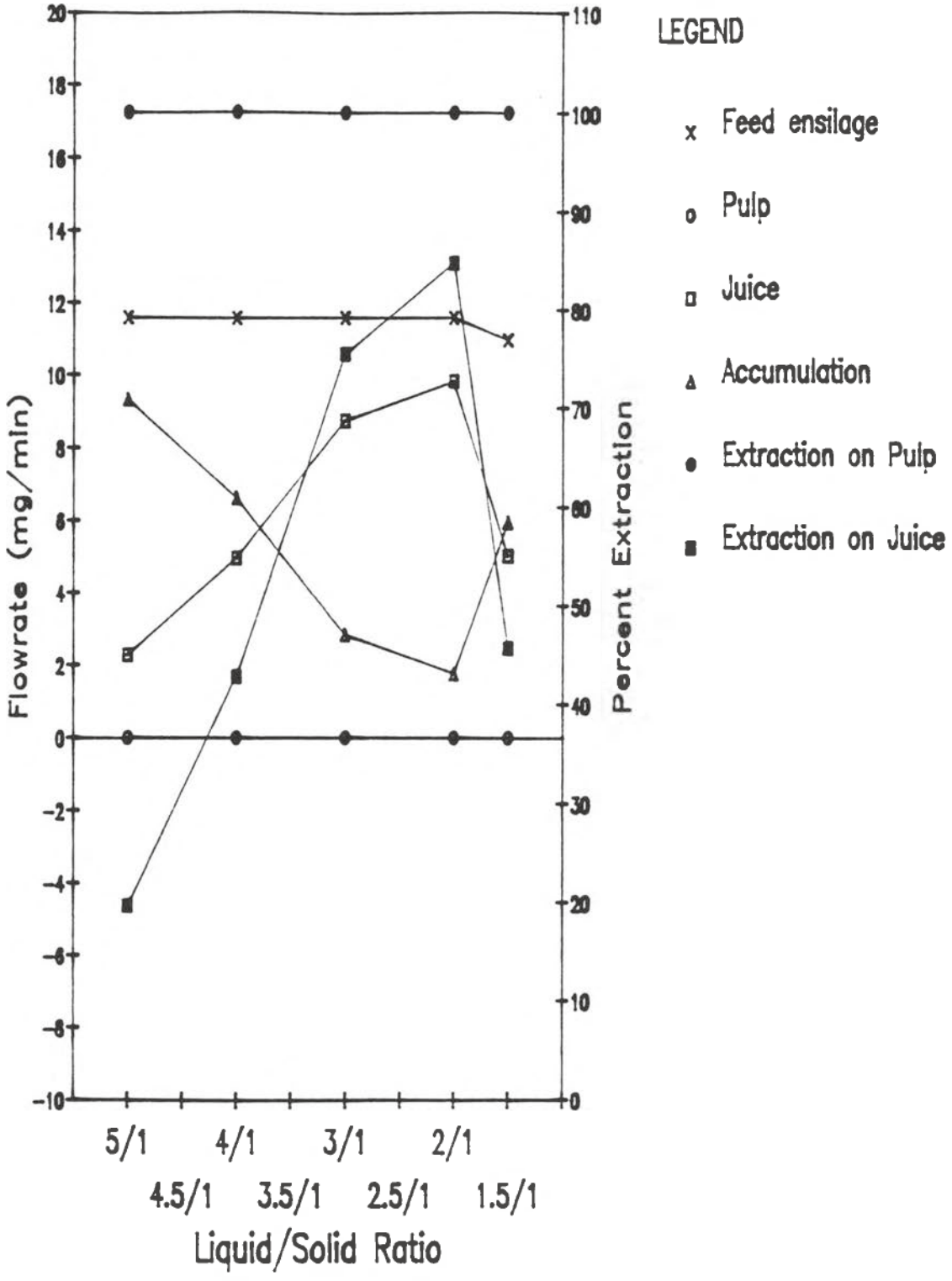


Figure 22G. Diffuser flowrates and extraction percentages for unknown #2 at 70°C.

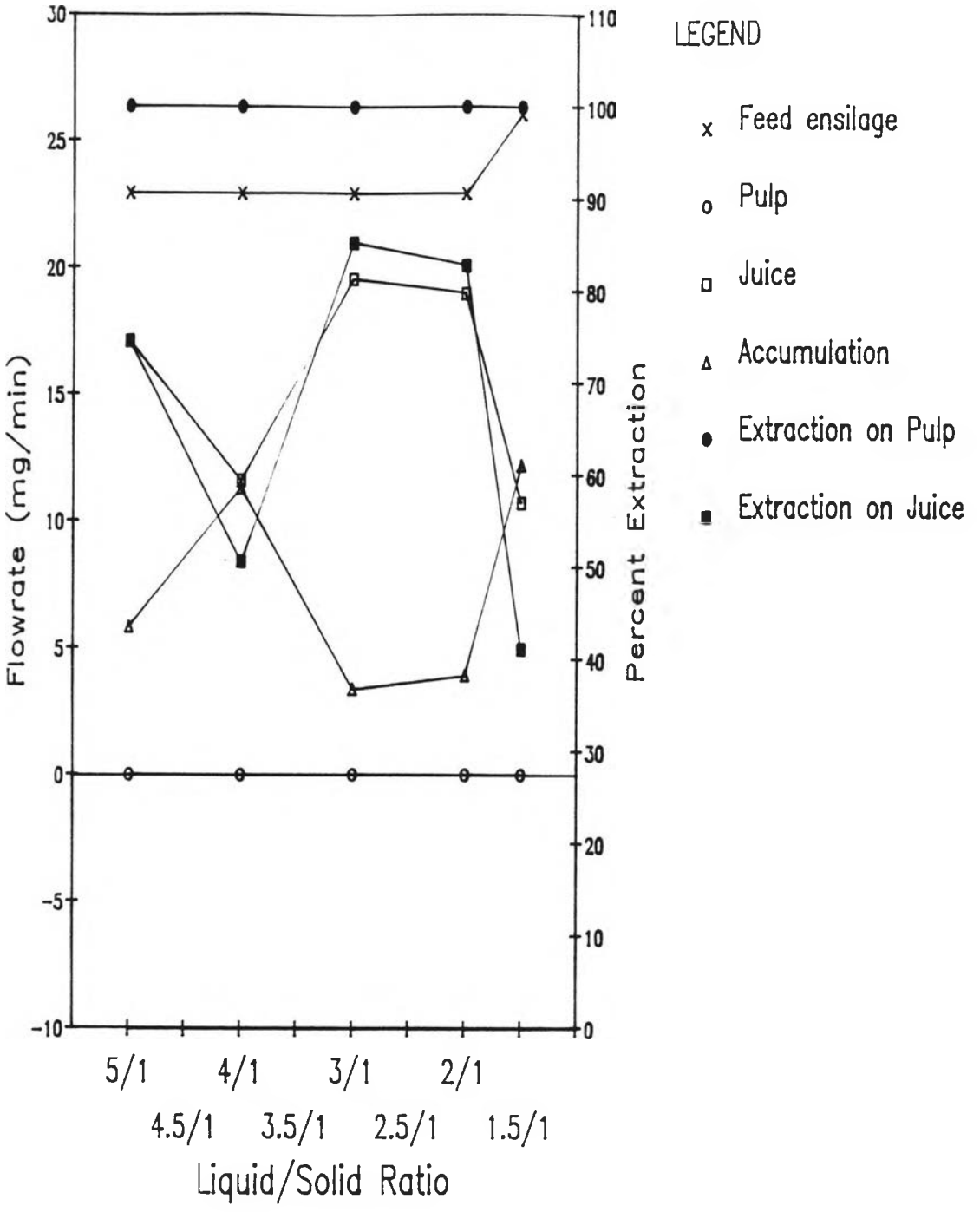


Figure 22H. Diffuser flowrates and extraction percentages for propionic acid at 70°C.

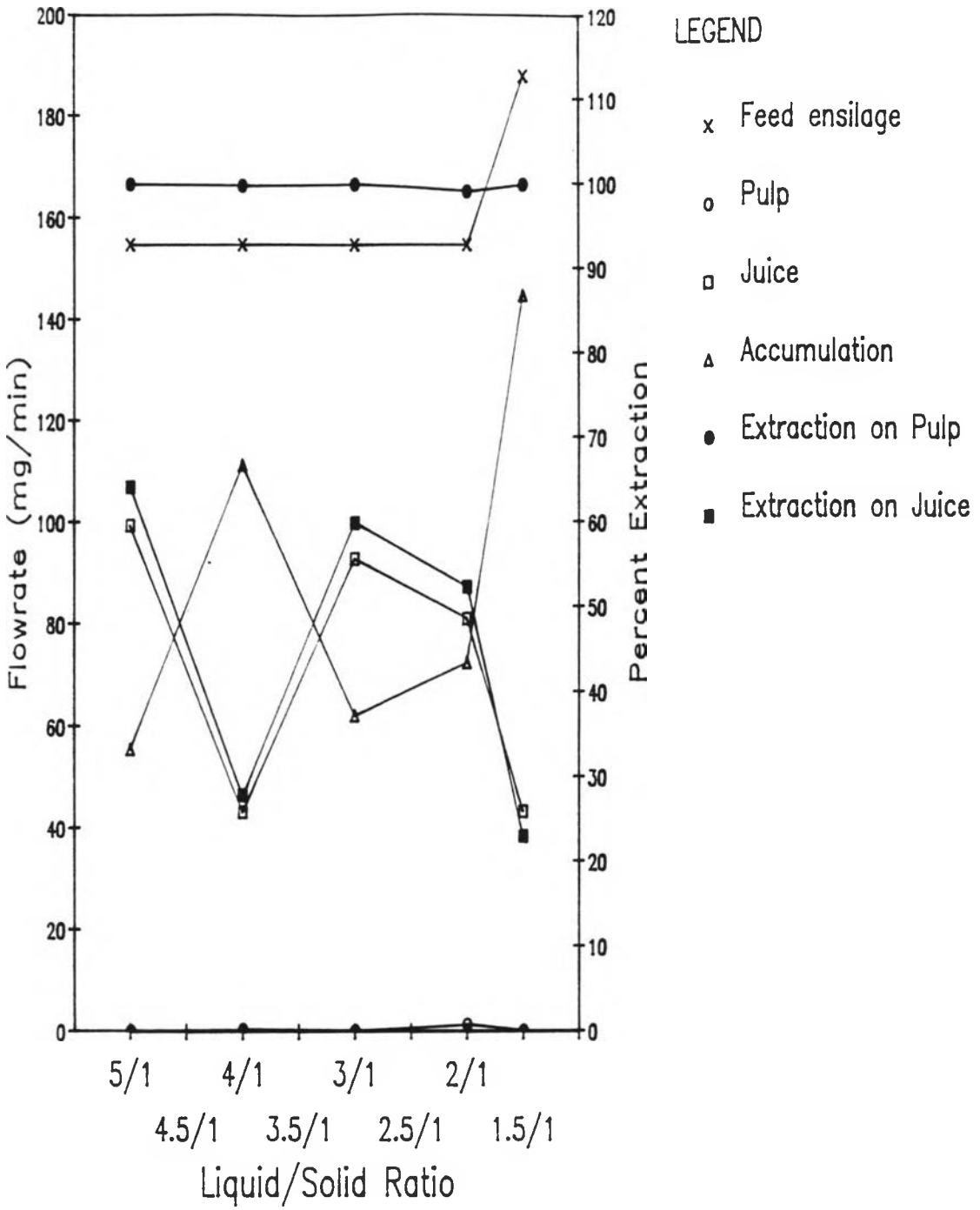


Figure 22I. Diffuser flowrates and extraction percentages for ethanol at 70°C.

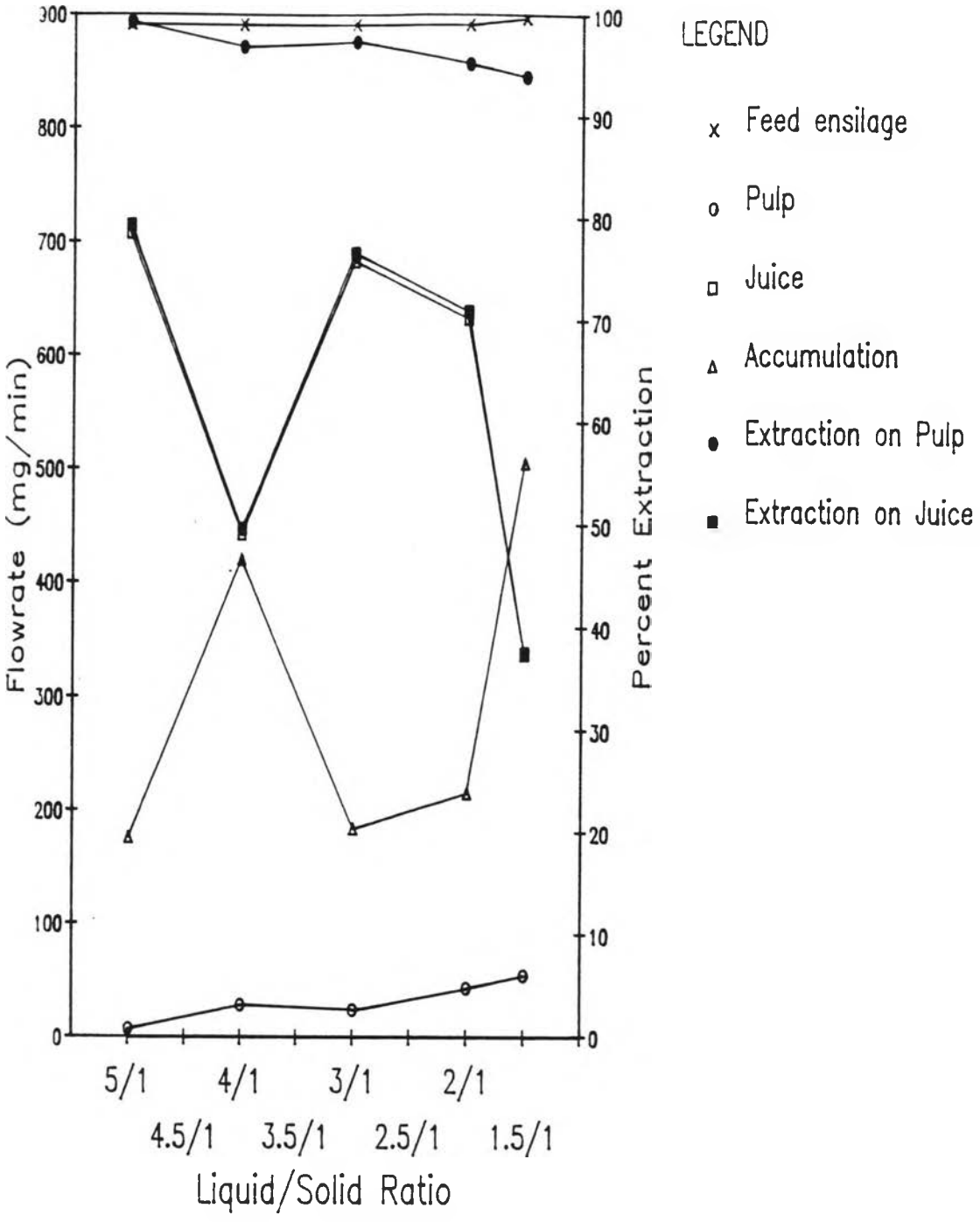


Figure 22J. Diffuser flowrates and extraction percentages for all components at 70°C.

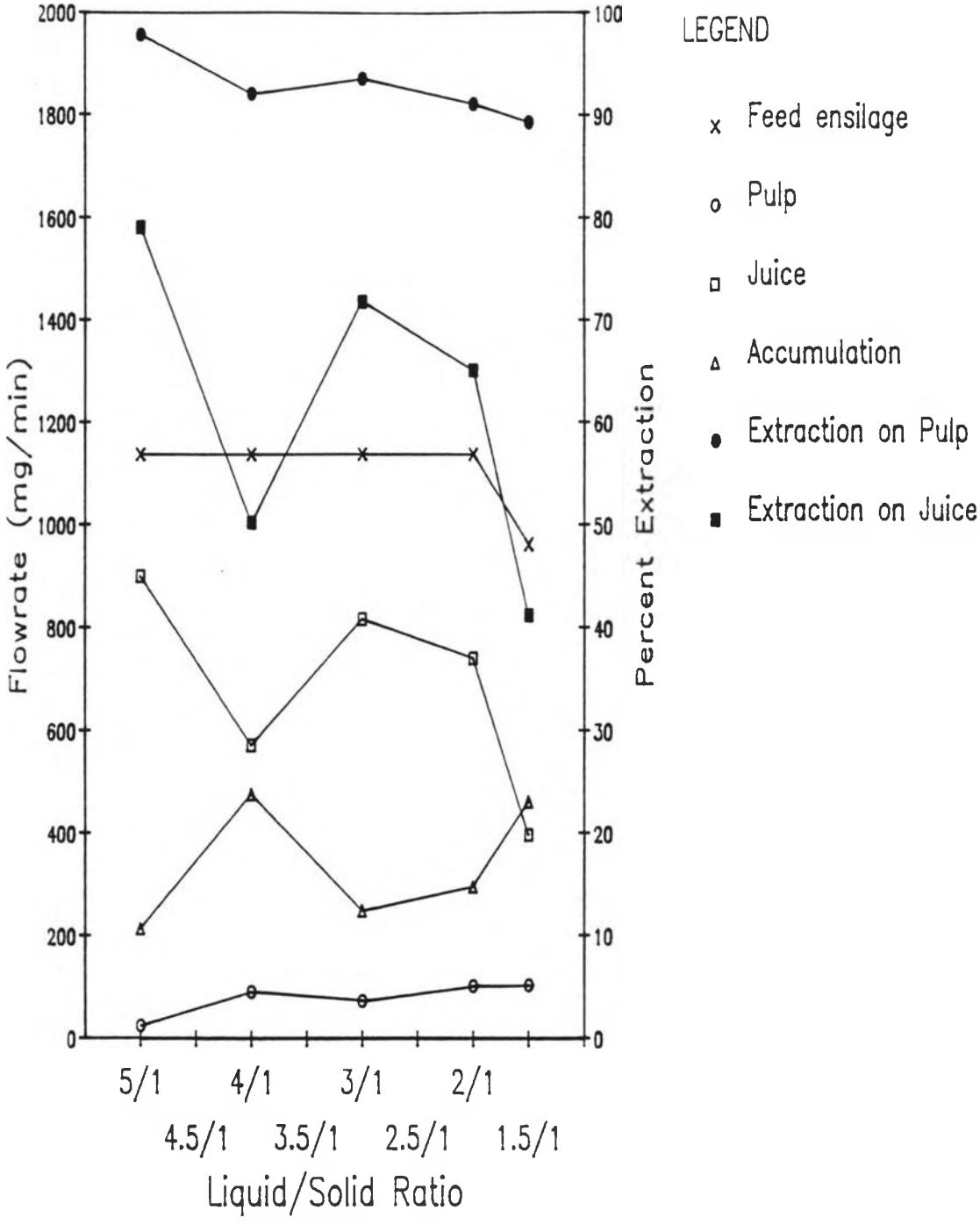


Figure 22K. Diffuser flowrates and extraction percentages for sugars at 70°C.

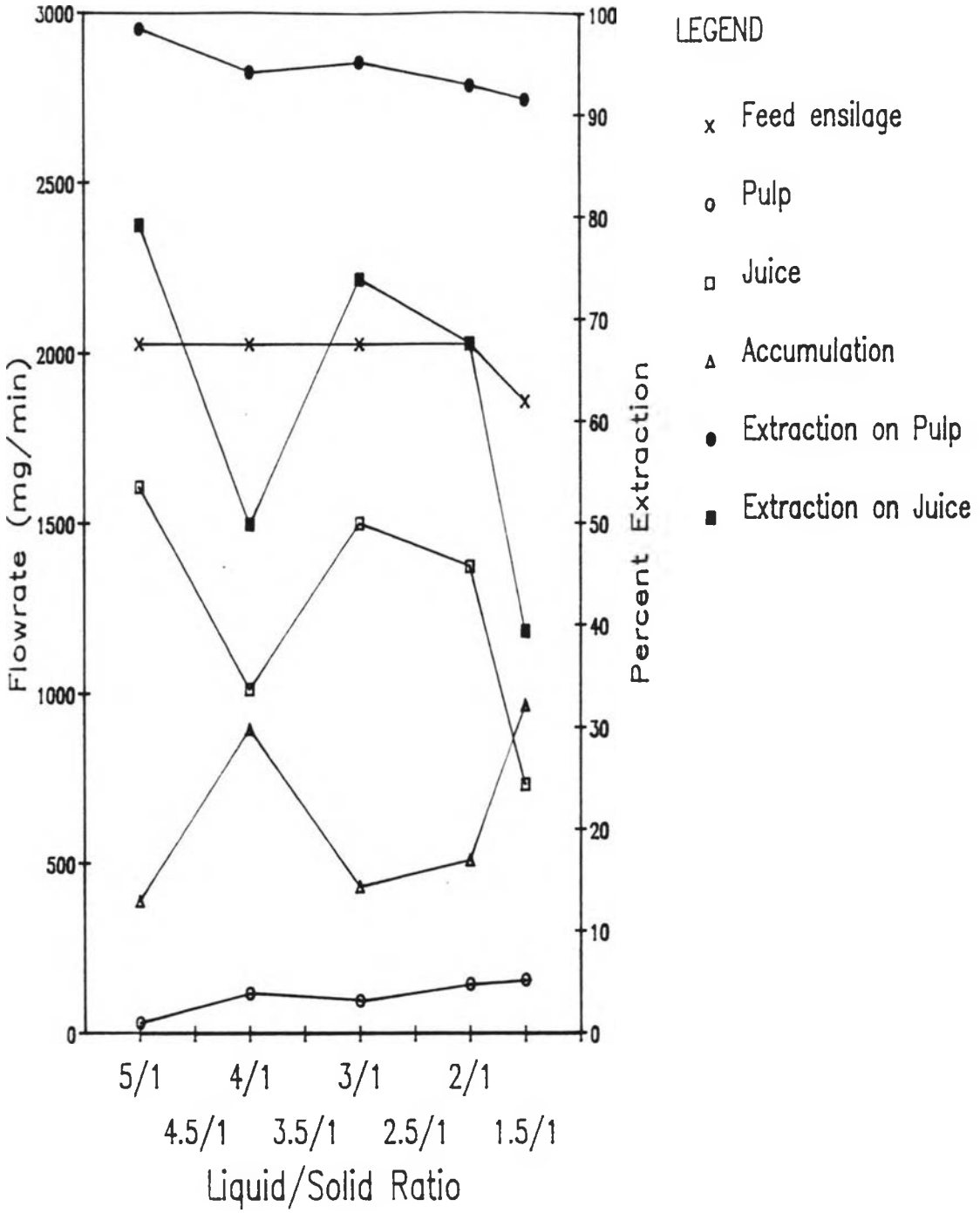


Figure 22L. Diffuser flowrates and extraction percentages for organic acids at 70°C.

curves (figs. 16 and 17). The exception was for fructose/arabinose combined HPLC analysis peak, total of all components, and sugars, at the 70°C 1.5 L/S ratio. For these components, the pulp concentration at this L/S ratio was lower than the pulp concentration at the 2.0 L/S ratio, but the mg/min was higher than the point at the 2.0 L/S ratio. The higher mg/min value was probably caused by the high dry matter value of the pulp for that run. The higher component total and sugar flowrate was obviously caused by the higher mg/min value for fructose/arabinose combined HPLC analysis peak.

Now looking at the extraction efficiency on pulp, the shape of the curve was inverse to the pulp flowrate for both the 50°C and 70°C runs. This was because of the way extraction on pulp was defined; the amount of a component removed from the feed ensilage ({FE-pulp}/FE), where FE represents the concentration of the component in the feed ensilage (equation 3). Therefore, if there was a high component pulp flowrate, there would be a corresponding low extraction on pulp since the component was not thoroughly removed from the pulp.

One thing common to the juice component flowrates for the 50°C runs was the high value for the 4.0 L/S run. This was caused by the high values for the component concentrations obtained from the 4.0 L/S run (fig. 18). One thing common to the juice component flowrates for the 70°C runs was the low component concentration values for the 4.0 and 1.5 L/S runs. The low values at the 4.0 L/S ratio were caused by the low values for the component concentrations obtained from that L/S ratio (fig. 19). The low values for the 1.5 L/S run were probably caused by the low overall juice flowrate at 70°C (fig. 20). In some instances the low component values at the 1.5 L/S run can also be

attributed to the drop in the ensilage component flowrate (sucrose/unknown #1, glucose, unknown #2, sugars). An exception to the low values for the 4.0 L/S run at 70°C was the unknown #2 component. For this component there was a steady increase in the the component flowrate from high to low L/S ratio. This was because of the steady increase in the unknown #2 component concentration for that L/S run (fig. 19). The low values at the 1.5 L/S ratio for unknown #2 can be attributed to the combination of the drop in the component feed ensilage rate and the overall low juice flowrate at that L/S ratio.

The unsteady nature of the juice component flow rate curves for both the 50°C and 70°C runs can be accounted for by the method of calculation to arrive at a component flowrate. Component flowrates were calculated by multiplying the overall juice mass flowrate (g juice/min) by the component concentration (mg X/g juice). From Figure 20, one can see that the overall juice flowrates drop with L/S ratio, while the component concentrations (figs. 18 and 19) generally increase with decreasing L/S ratio. Multiplying a decreasing number by an increasing number will create an unsteady nature in the product, and thus helped explain the nature of the juice component flowrates.

Looking at the extraction efficiency based on juice, one sees that the shape of the curve (for example, see fig. 22K) followed the shape of the curve for the juice component flowrates for both temperatures. This was because of the definition of extraction efficiency based on juice: the amount of a component in the juice divided by the amount of the same component originally in the feed ensilage (equation 4). Therefore, a drop in a juice component flowrate decreased the extraction efficiency based on juice. Occasionally for the 50°C

runs, a juice component flowrate was found to be greater than the feed ensilage component flowrate; especially for the 4.0 L/S ratio where it happened for 7 out of the 9 components (not for stachyose/raffinose or ethanol). This was probably due to HPLC error giving high concentrations for those components (fig. 18). The juice component flowrates greater than the feed ensilage component flowrates caused the greater than 100 percent values for the extraction efficiency based on juice. It was interesting to note that there were no extraction efficiencies based on juice greater than 100 percent for the 70^{o}C runs.

The accumulation terms followed the inverse shape of the juice flowrates for both temperatures. This was the opposite for the accumulation terms for the overall flowrates, which followed the inverse shape of the pulp flowrates. The negative accumulation terms for components during the 50^{o}C runs correspond to when the juice component flowrates where greater than the feed ensilage component flowrates. The pulp component flowrates varied very little with L/S ratio while the juice component flowrates varied greatly with L/S ratio. Therefore, it makes sense that the accumulation terms were influenced by the juice component flowrates.

Temperature had little effect on the percent extraction of a component removed from the pulp. Occasionally, the percent extraction on pulp for a given component was greater for the 70^{o}C runs than for the 50^{o}C runs. In fact, the percent extraction based on pulp never dropped below 90 percent except for the 70^{o}C 1.5 L/S values for sucrose/unknown #1 (87%) and glucose (82%). Remember these low values were a result of the fact that less water was available for extraction

at low L/S ratios. A smaller concentration gradient was created which allowed less diffusion to occur as the L/S dropped. Temperature also had little effect on the extraction based on juice.

Another important point must be made. The extraction efficiency based on pulp was for the majority of cases greater than the extraction efficiency based on juice for both temperatures. The exceptions were the large extraction percentage values for the juice 50°C 4.0 L/S runs and for unknown #2 at the 50°C 5.0 L/S run as explained earlier. If the diffuser was at steady state, when 90 percent of a component was removed from the feed ensilage (extraction based on pulp), 90 percent should be in the juice. The lower values for the extraction efficiency based on juice than on pulp signify that the diffuser was not at steady state. Part of the reason for the unsteady state condition was that a run lasted around 1 hour, whereas the retention time of the sorghum was approximately 90 to 120 minutes. Therefore, juice samples were being collected before the sorghum could travel the length of the diffuser. Another possible explanation may be that some components evaporated while traveling the diffuser, especially the organic acids. Also the sugars may have been consumed by microbiological activity in the diffuser and therefore helped account for the accumulation terms. The unsteady nature also helped account for the accumulation terms.

The unsteady state nature of the diffuser was also shown by the data for run 10. Run 10 was conducted at the 1.5 L/S ratio and 70°C. It was the only run for the day and lasted approximately 7 hours. It consisted of 11 subruns. Data was collected every 30 minutes after first allowing operation for 2.5 hours. Figure 23 shows

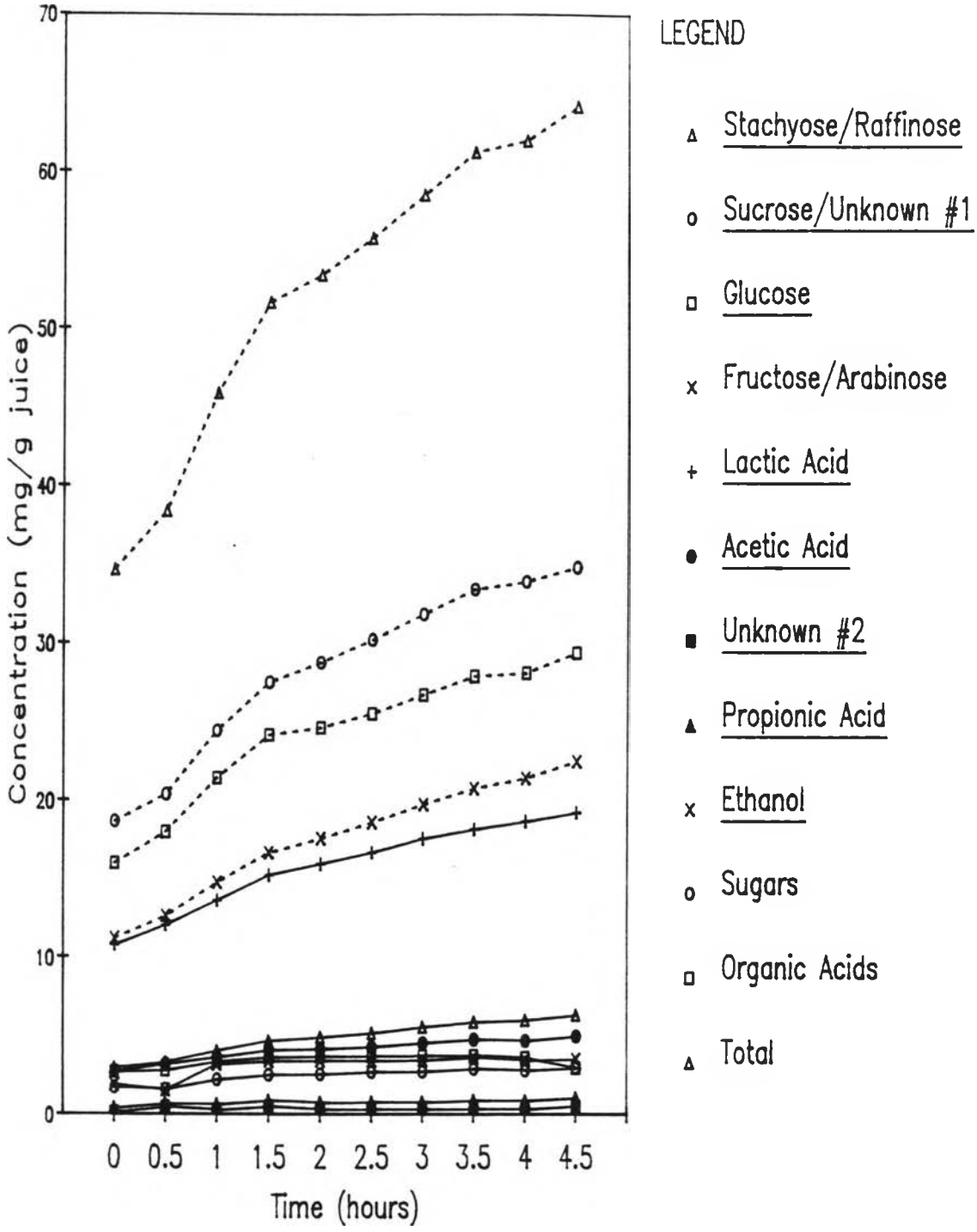


Figure 23. Juice component concentrations for run 10.

the juice component concentrations for the run as a function of time, while Figures 24 A-L show the flowrates and extraction efficiencies for the individual components. From Figure 23 one sees that the juice concentration kept increasing as the run progressed even though the juice flowrate was unsteady. Figures 24 A-L show that it was the unsteady nature of the juice and pulp component flowrates which cause the corresponding unsteady nature of the accumulation and juice extraction efficiency terms. If the system had been in steady state, one would have seen a level juice concentration and fixed juice and pulp flowrates.

A theoretical treatment of the continuous countercurrent diffusion process was attempted by using the mathematical treatment of diffusion proposed by Silin (56). The mathematical treatment of diffusion proposed by Silin was developed for the continuous countercurrent diffusion of sugar from sugar beets. McGinnis (40) also applied and explained Silin's treatment of diffusion for the continuous countercurrent diffusion of sugar beets.

Silin started with Fick's first law which he writes as:

$$dS = D\ A\ dC/dr\ dt \tag{5}$$

where dS is the weight of the dissolved substance diffusing through area A in time dt, dC/dr is the concentration gradient of the dissolved substance, and D is the diffusion coefficient of the substance in question.

Let C_0 represent the concentration of sugar in the juice within the sweet sorghum ensilage entering the diffuser, C_n the concentration of sugar in the juice within the outgoing pulp, and C the concentration of sugar in the juice within the sweet sorghum ensilage

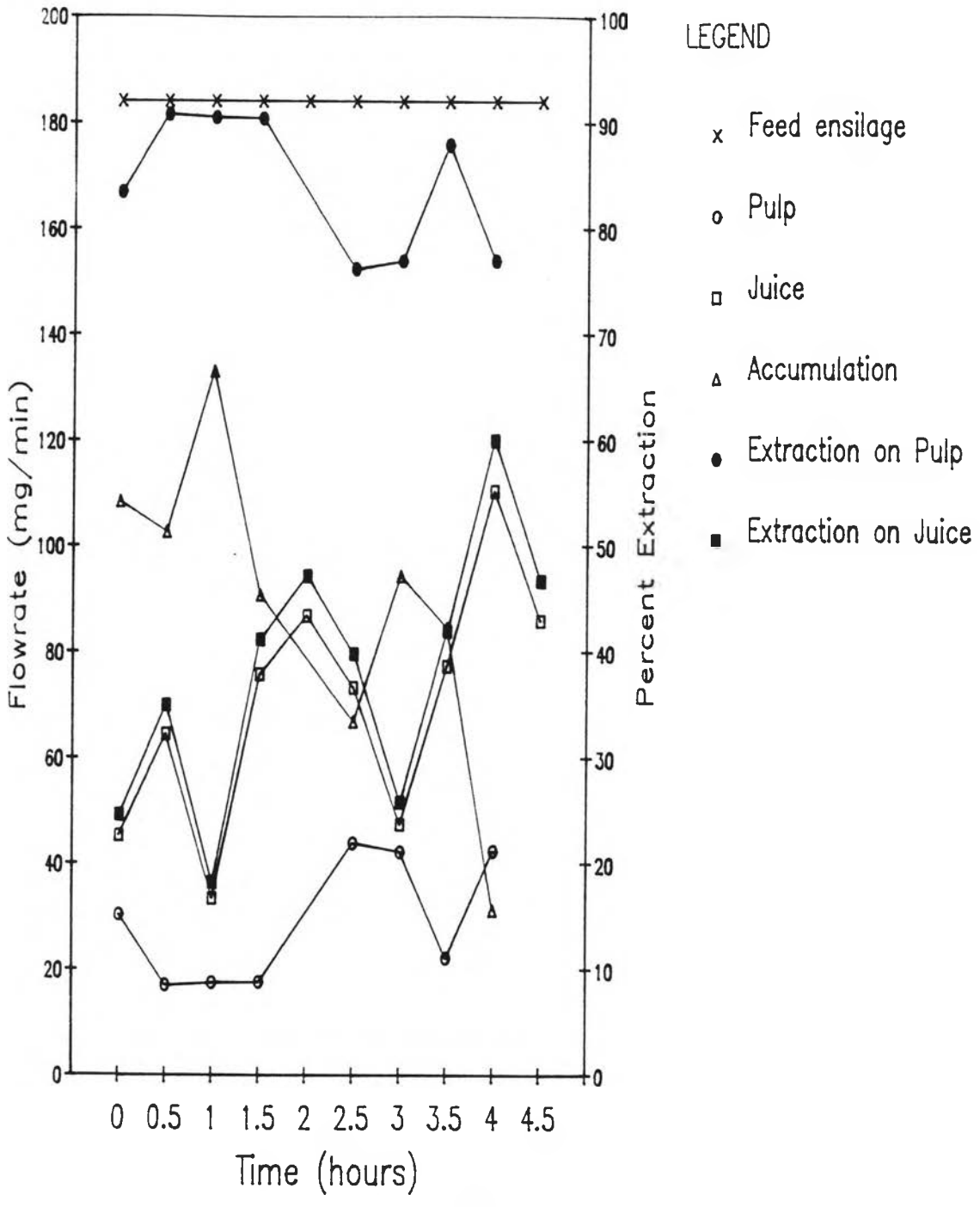


Figure 24A. Diffuser flowrates and extraction percentages during run 10 for stachyose/raffinose. First data point at 2.5 hrs (0 hrs in figure).

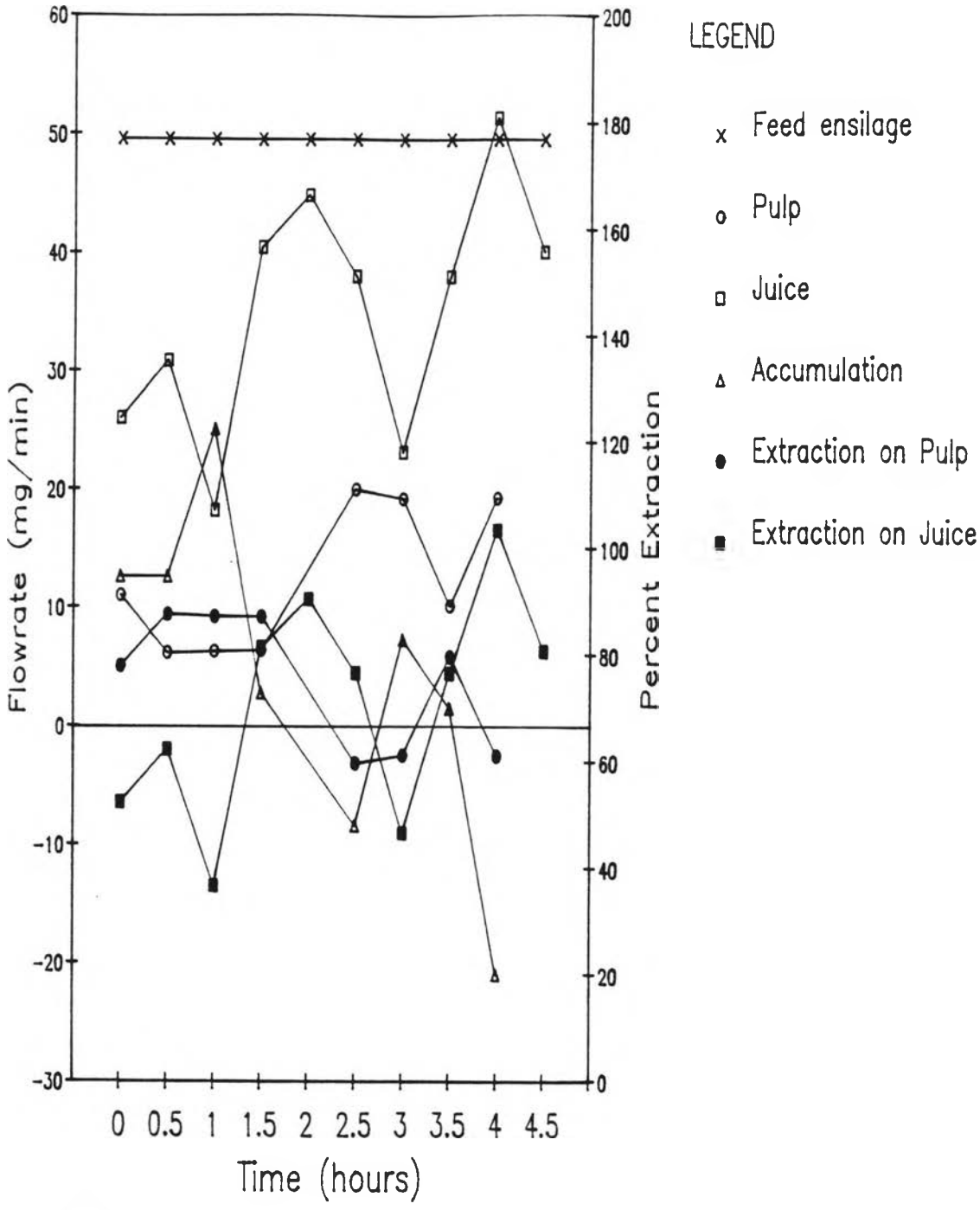


Figure 24B. Diffuser flowrates and extraction percentages during run 10 for sucrose/unknown #1. First data point at 2.5 hrs (0 hrs in figure).

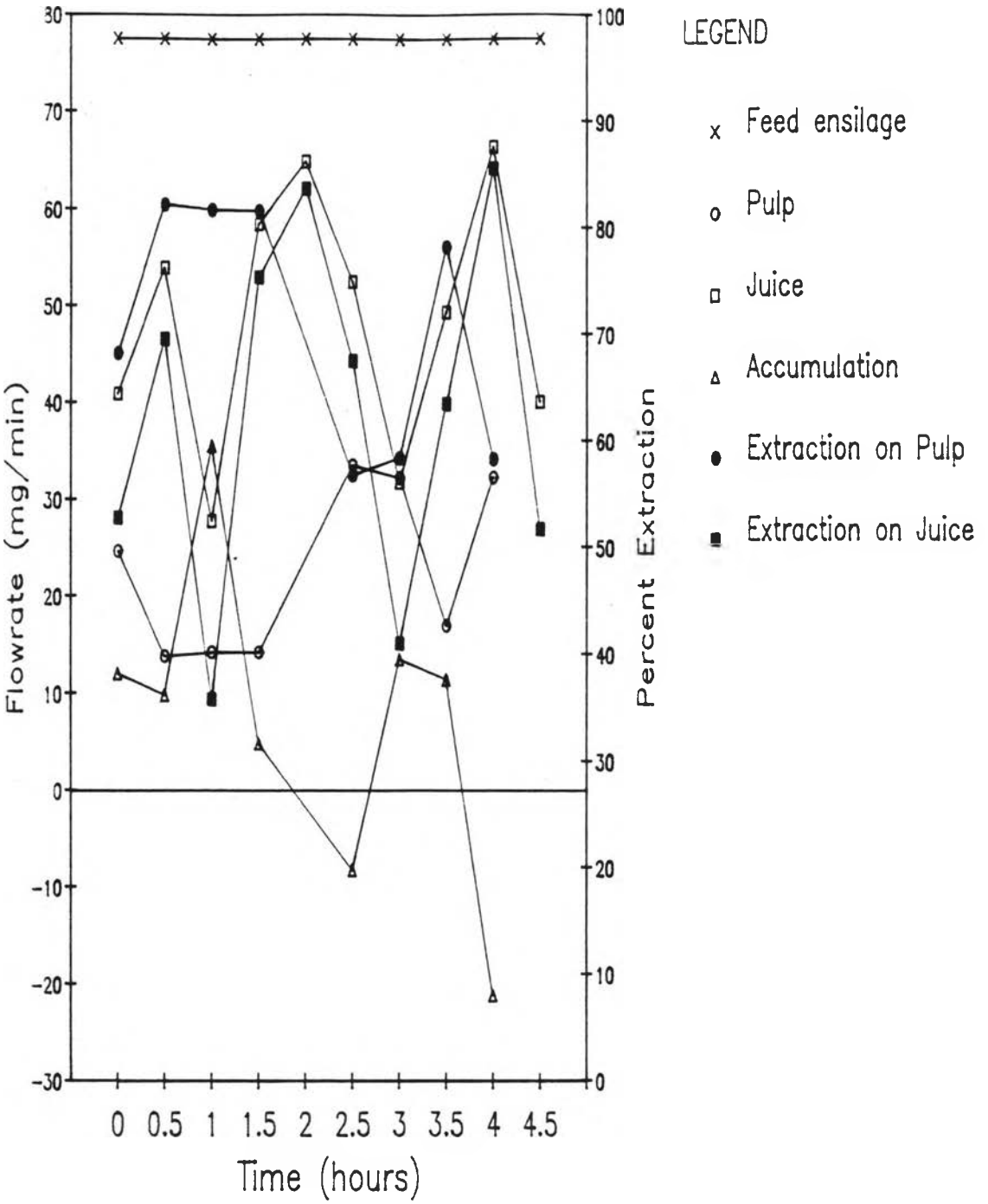


Figure 24C. Diffuser flowrates and extraction percentages during run 10 for glucose. First data point at 2.5 hrs (0 hrs in figure).

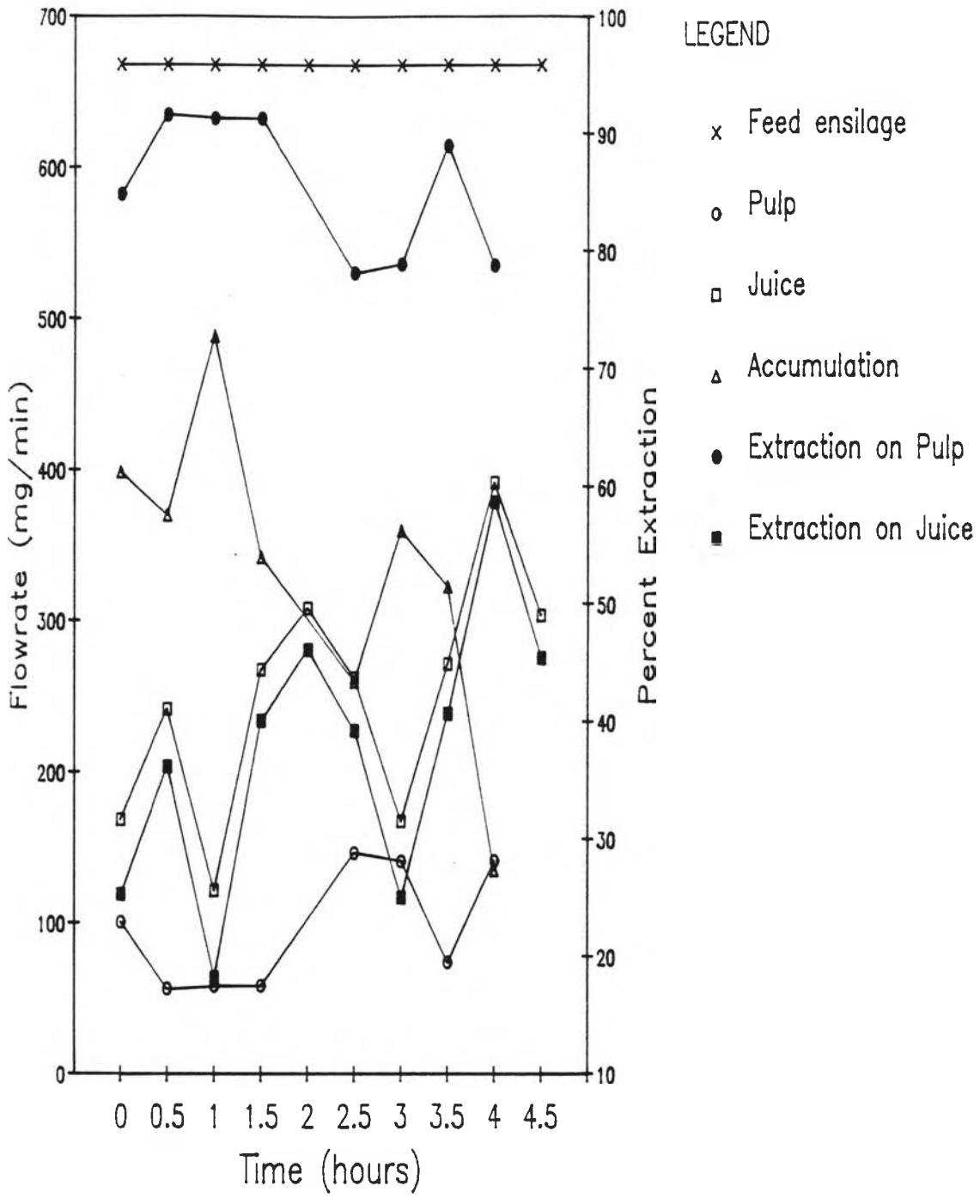


Figure 24D. Diffuser flowrates and extraction percentages during run 10 for fructose/arabinose. First data point at 2.5 hrs (0 hrs in figure).

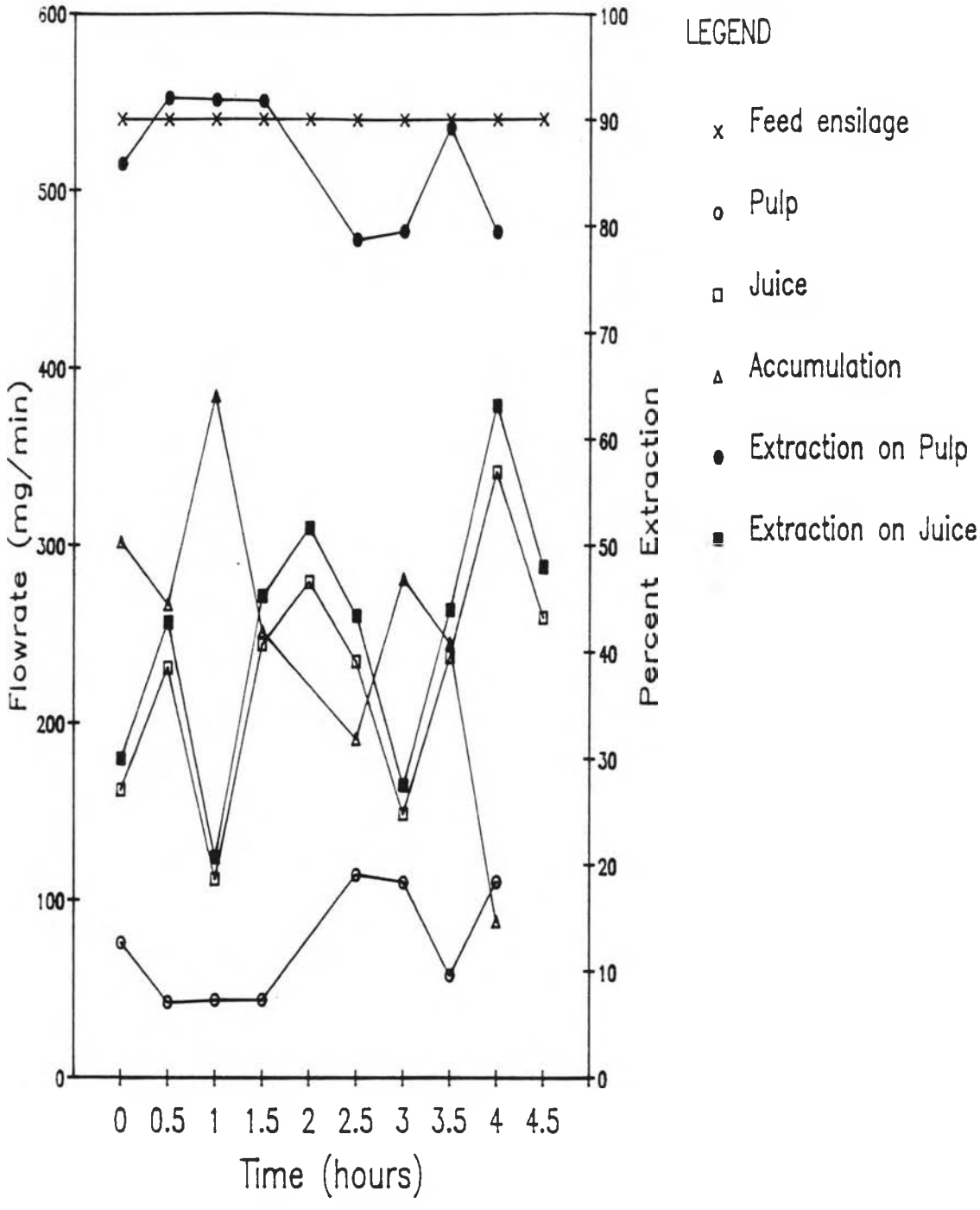


Figure 24E. Diffuser flowrates and extraction percentages during run 10 for lactic acid. First data point at 2.5 hrs (0 hrs in figure).

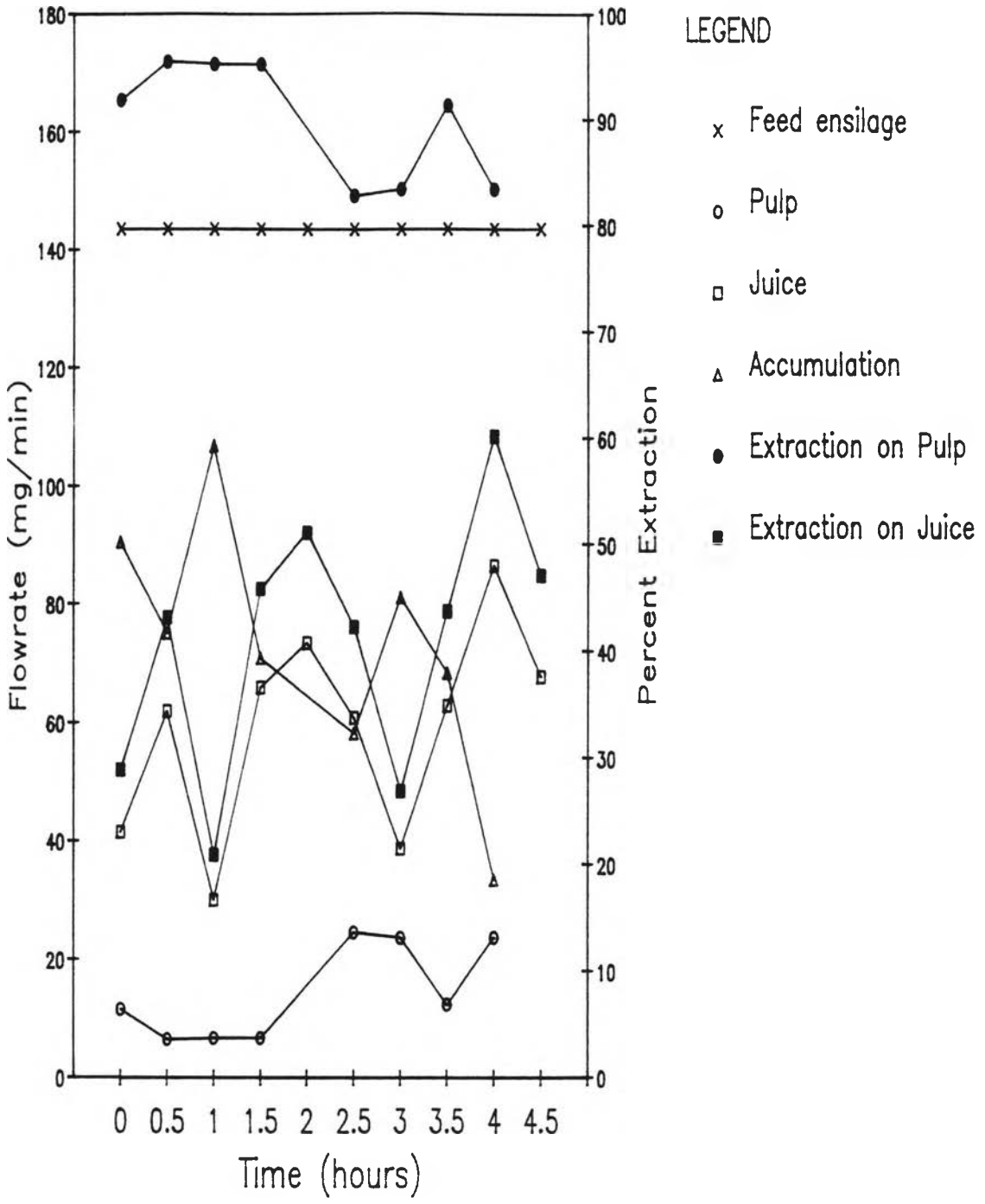


Figure 24F. Diffuser flowrates and extraction percentages during run 10 for acetic acid. First data point at 2.5 hrs (0 hrs in figure).

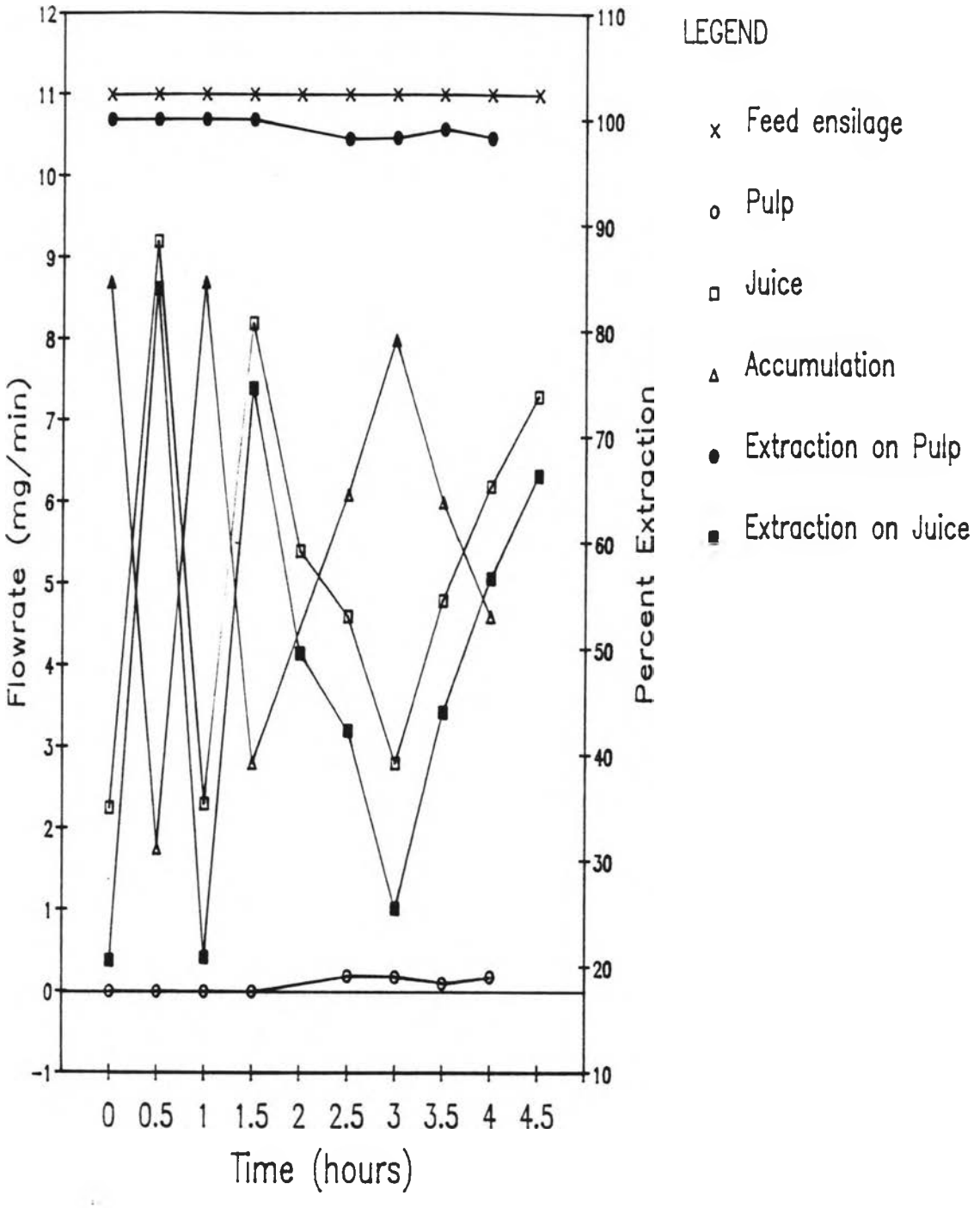


Figure 24G. Diffuser flowrates and extraction percentages during run 10 for unknown #2. First data point at 2.5 hrs (0 hrs in figure).

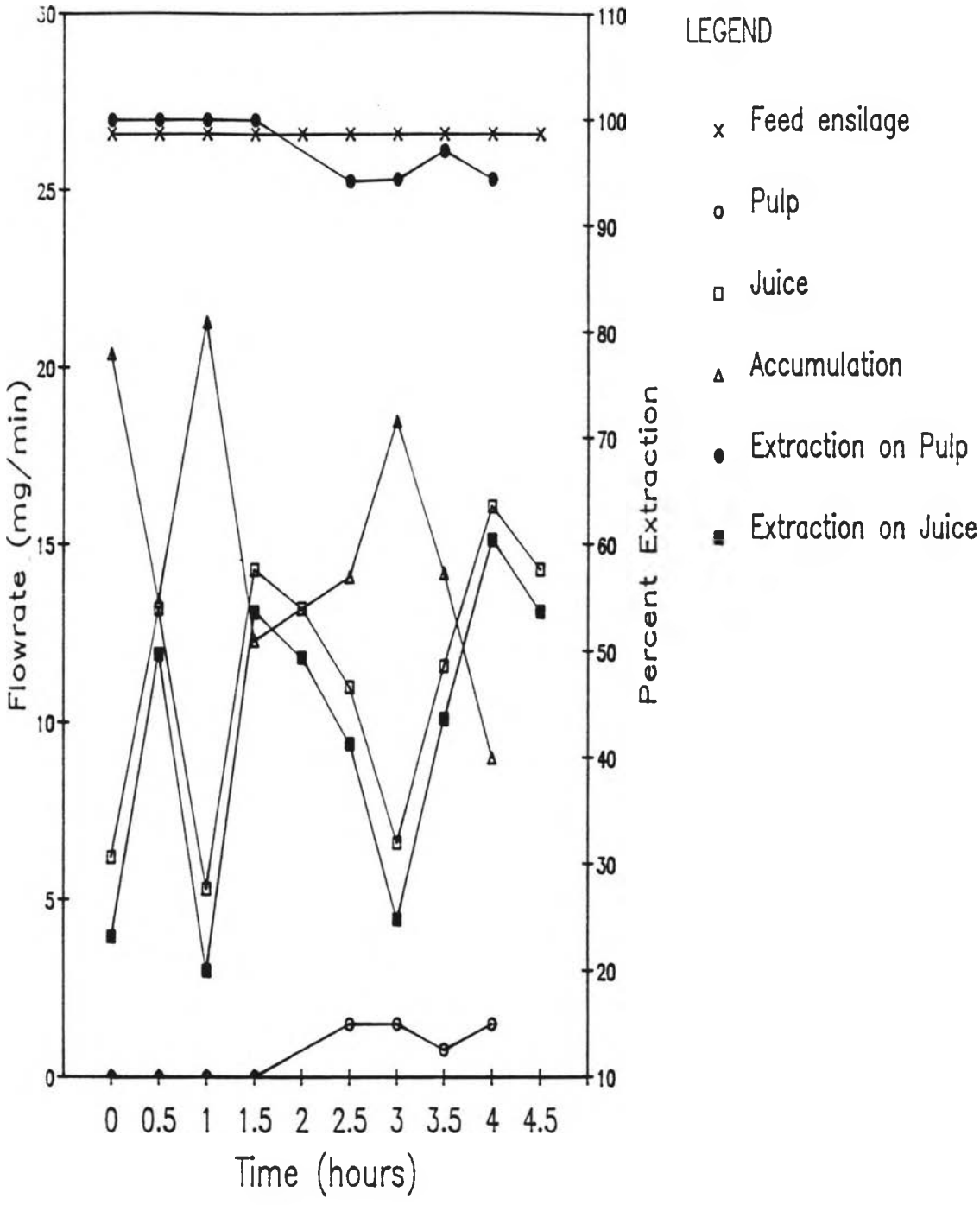


Figure 24H. Diffuser flowrates and extraction percentages during run 10 for propionic acid. First data point at 2.5 hrs (0 hrs in figure).

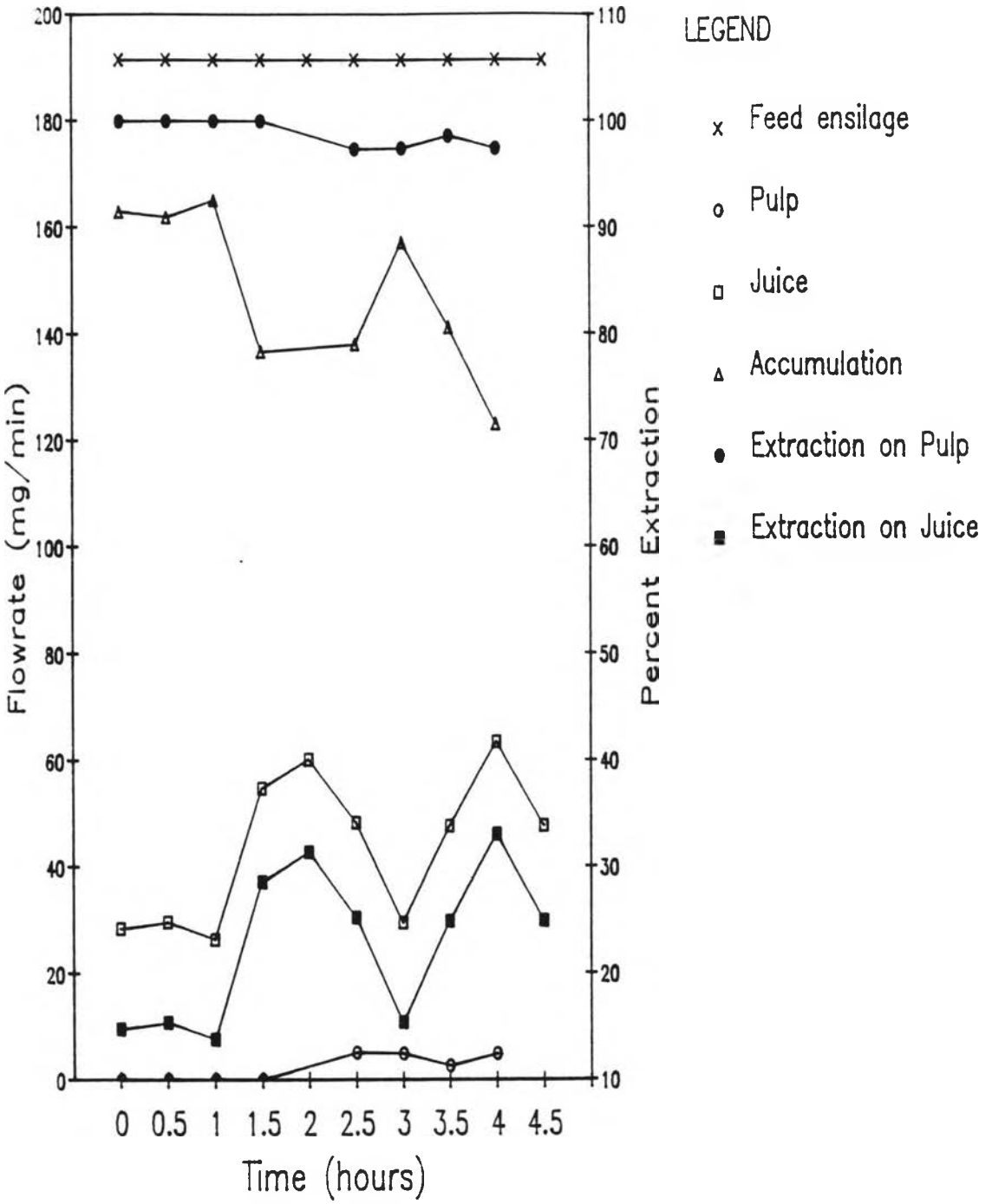


Figure 24I. Diffuser flowrates and extraction percentages during run 10 for ethanol. First data point at 2.5 hrs (0 hrs in figure).

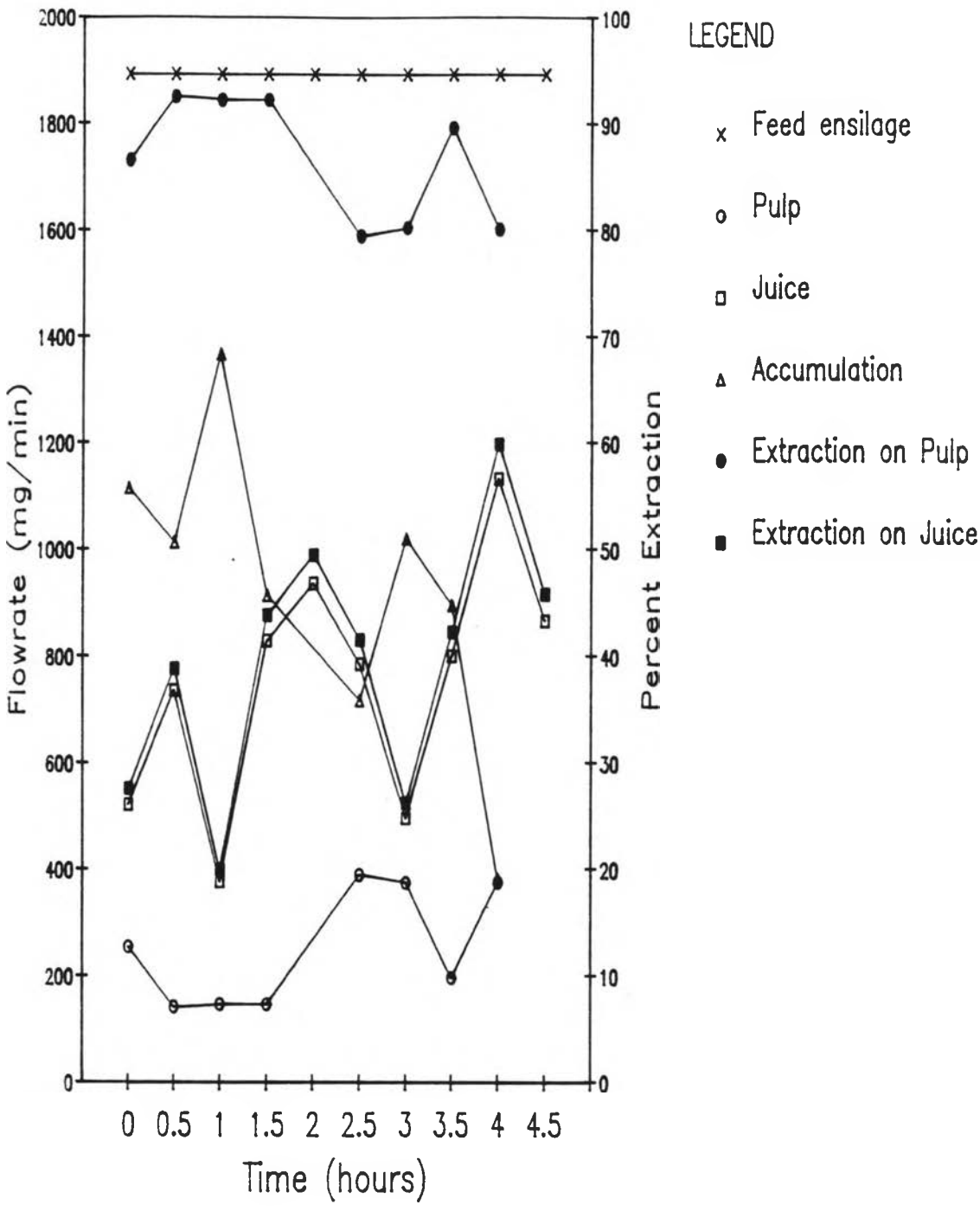


Figure 24J. Diffuser flowrates and extraction percentages during run 10 for all components. First data point at 2.5 hrs (0 hrs in figure).

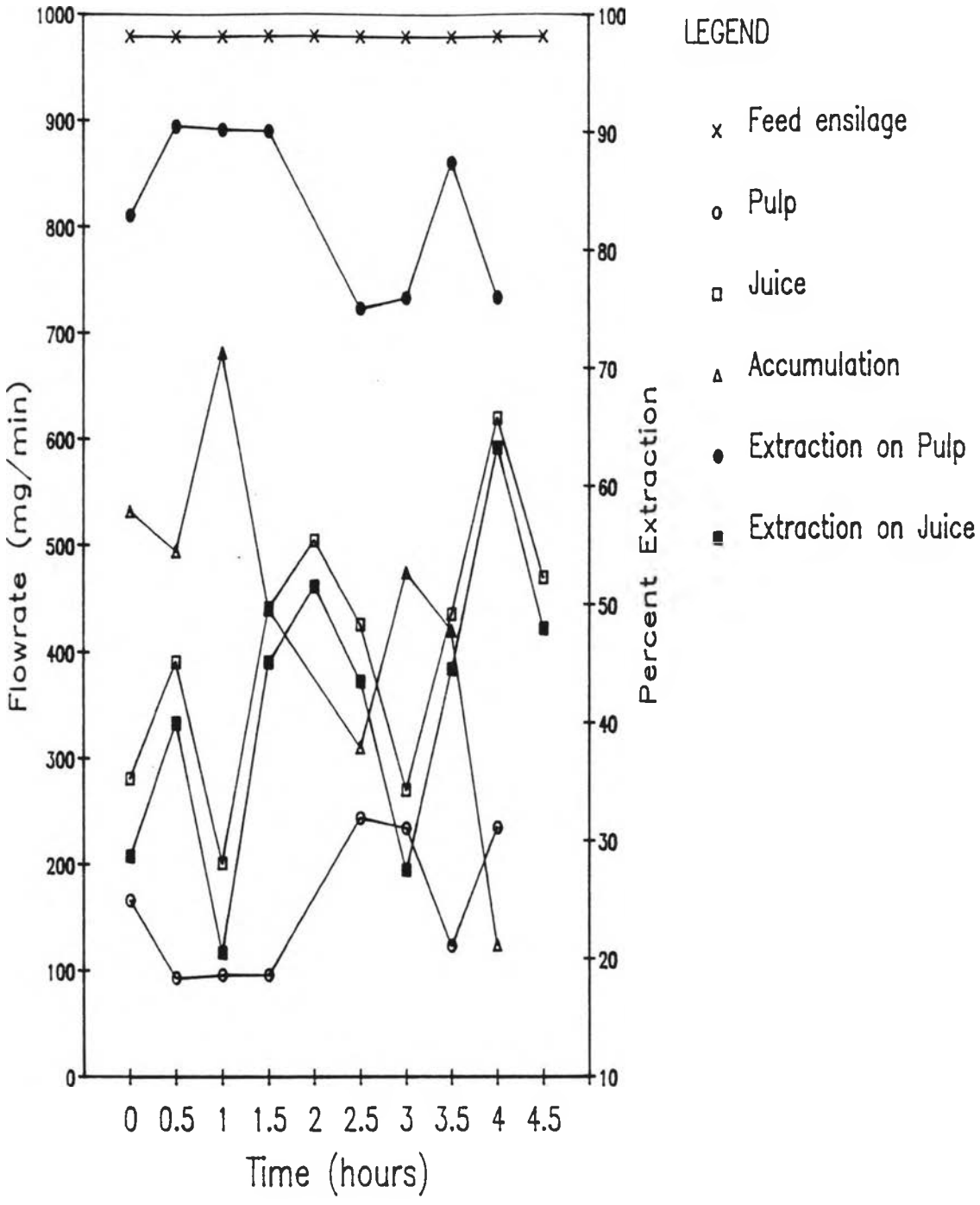


Figure 24K. Diffuser flowrates and extraction percentages during run 10 for sugars. First data point at 2.5 hrs (0 hrs in figure).

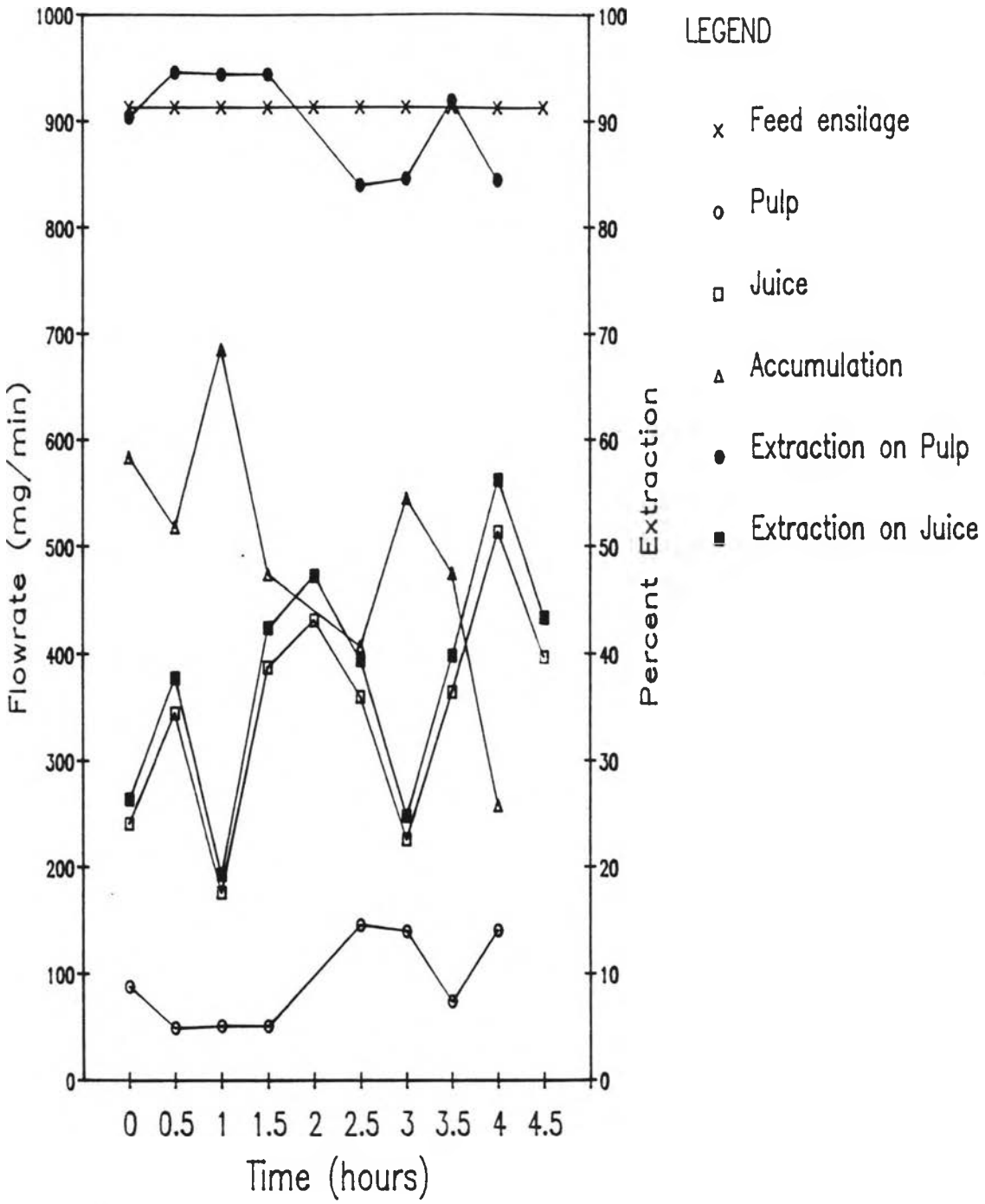


Figure 24L. Diffuser flowrates and extraction percentages during run 10 for organic acids. First data point at 2.5 hrs (0 hrs in figure).

at any intermediate point. Similarly let J_o be the concentration of sugar in the raw juice, J_n the concentration of sugar of the supply water, and J the concentration of sugar of the juice surrounding the sweet sorghum ensilage. All concentrations are expressed as weight percent on juice.

Assuming steady state and cylindrical pieces of sweet sorghum, the concentration gradient dC/dr can be considered proportional to (C-J)/0.5r (the average distance of diffusion through the cylindrical sorghum is half the radius). The length of the cylindrical sorghum was assumed to be much longer than its diameter so that diffusion through its end surfaces may be disregarded. The average (C-J) through the length of the diffuser can be determined by calculating the logarithmic mean difference:

$$C - J = \frac{(C_o - J_o) - (C_n - J_n)}{\ln (C_o - J_o)/(C_n - J_n)} = \frac{C_o - J_o - C_n}{\ln (C_o - J_o)/C_n} \qquad (6)$$

Here J_n was assumed to be zero. Equation (5) now becomes:

$$dS = \frac{D\ A\ (C_o - J_o - C_n)\ p_{js}}{\ln[(C_o - J_o)/C_n]\ 0.5r}\ dt \qquad (7)$$

where the concentration gradient (dC/dr) was multiplied by the density of the juice in the sorghum (p_{js}). Silin (56) and McGinnis (40) do not mention a density being used in their equations, but one was needed to balance the units.

In a continuous diffuser, all the sugar diffused from the sorghum in the diffusion time is gained by the juice. Therefore, the amount diffused from a unit weight of juice within the sorghum will be (C_o-C_n). The basis or unit weight of juice chosen by Silin is

100 kg of juice within the sorghum. Now equation (7) becomes:

$$C_o - C_n = \frac{2\ D\ A'}{r}\ \frac{t\ (C_o - J_o - C_n)\ p_{js}}{\ln[(C_o - J_o)/C_n]} \qquad (8)$$

where A′ is now the area of that quantity of sorghum that contains 100 kg of juice.

The juice produced with a concentration J_o contains all the sugar extracted from the sorghum, that is, per unit weight of juice in the sorghum (C_o-C_n). If n parts of diffusion juice are produced per unit weight of juice within the sorghum, then $nJ_o = C_o - C_n$. Substituting this into equation (8) gives upon rearranging:

$$n/(n - 1)\ \ln\{[(n - 1)C_o + C_n]/nC_n\} = 2\ D\ (A'/r)\ t\ p_{js} \qquad (9)$$

Solving equation (9) for C_n:

$$C_n = \frac{C_o\ (n - 1)}{n\ \exp\{[(n - 1)/n]\ 2\ D\ (A'/r)\ t\ p_{js}\} - 1} \qquad (10)$$

The values A′ and r in equation (10) are the surface area of sorghum containing 100 kg of juice and the mean radius, respectively. They both depend on the length of a given weight of sorghum, and their ratio is proportional to the Silin number (L), which is defined as the length in meters of 100 g of sorghum. Thus, A′ and r are functions of L. The larger the L, the thinner the sorghum, the smaller the r and the greater the value of A′.

The length of the quantity of sorghum that contains 100 kg of juice is 1000 L/Z where Z is the juice coefficient, the amount of juice within the sorghum on a weight percent basis. As Silin presupposes cylindrical coordinates, the surface area that contains 100 kg of juice can be

expressed as:

$$A' = 2 \pi r (1000 L/Z) \text{ [m/100 kg juice]} \quad (11)$$

or

$$A'/r = 200 \pi L/Z \text{ [cm/100 g juice]} \quad (11a)$$

Therefore, equation (10) becomes:

$$C_n = \frac{C_o (n - 1)}{n \exp\{[(n - 1)/n] 400 \pi D (L/Z) t p_{js}\} - 1} \quad (12)$$

Defining H as a constant, $H = 2 D (A'/r) t p_{js}$, it was shown that by using the Silin number (L) to find an expression for A'/r that:

$$H = 400 \pi (L/Z) D t p_{js} \quad (13)$$

But Silin goes furthur in defining H. First he uses Einstein's correlation for D:

$$D = k_o T/n \quad (14)$$

where k_o is a constant for the dissolved substance indepenent of temperature but dependent on particle size of the solute, T is the absolute temperature, and n the viscosity of the solvent at a given temperature. Since T and n are both functions of temperature, the ratio can be set proportional to a new variable θ, the viscosity factor:

$$\theta = T/(1000 n) \quad (15)$$

Silin and McGinnis define θ as ^oK cm s/g in tables using n in poise. Substituting equation (15) into equation (14) makes $D = 1000 k_o \theta$. Silin uses A' as expressed in equation (11). Therefore, $H = 4 k_o 10^6 (\pi/Z) \theta L t p_{js}$. He now lets a constant K equal $4 k_o 10^6 (\pi/Z)$, which is influenced by the material being extracted and the diffuser design. Substituting K into the equation

for H:

$$H_K = K\ \theta\ L\ t\ p_{js} \tag{16}$$

Silin conducted experiments to determine K under widely different conditions, and found K had an average of 6.5×10^{-5}. This value however was calculated using equation (9) in which concentration was expressed as logs instead of ln (i.e. H would have a 1/2.302 on the right hand side of the equation) and at an assumed juice coefficient of 0.93. Therefore, the K that should be used here is:

$$K = 2.302\ (0.93/Z)\ K_{Silin} \tag{17}$$

Recall, C_n is the weight percent of sugar in the juice within the pulp leaving the diffuser and C_o is the weight percent of sugar in the juice within the entering sorghum. From equation (6)

$$C_n = \frac{C_o\ (n - 1)}{n\ \exp[\{(n - 1)/n\}\ H] - 1} \tag{18}$$

where H is defined by equation (13) or equation (16). In equation (13) and (16) D is the diffusion coefficient in cm^2/min, t is the time in minutes of the diffusion, p_{js} is the density of the juice within the entering sorghum to the diffuser in g/cm^3, r is the radius of a particle in cm, Z is the juice coefficient (g juice within the sorghum/g sorghum), L is length in meters of 100 g of sorghum, n is the g juice/g juice within the sorghum, and K is a constant for the diffuser.

To calculate the Silin number (L) for the ensiled chopped sweet sorghum used in my experiments, a representative sample was split into 7 sections; fine mesh, little leaves, little stalks, medium stalks, large stalks, large leaves and seed heads. Each section was weighed and a Silin number was measured for each section. The average Silin

number was calculated by adding together the weighted Silin number of each section:

$$L = \Sigma \text{ (weight percent)}_i \, L_i \qquad (19)$$

The results for each section can be seen in Table 5. The overall average Silin number was 27.72 $\pm$ 6.82 m/100 g of sorghum.

From Table 6 one can see that substituting in H or H_K into equation (18) makes the exponential term either unbounded or ridiculously large, such that C_n would be zero no matter the value of the L/S ratio or C_o. The terms for H_K assume that the necessary conversion factors for units to cancel were concealed in the Silin constant, $K_{Silin} = 6.5 \times 10^{-5}$. Also, the values for H and H_K were calculated with an overall Silin number which includes the leaves and seed heads. Generally, the leaves and seed heads contain very little sugar as compared to the stalks. Therefore, a Silin number for stalks would be better to calculate the H and H_K values. Using just the stalks, and noting that the sugars in the ground stalks within the fine mesh were neglected, the stalk Silin number is 3.12 $\pm$ 0.62 m/100 g of sorghum. This was 8.9 times less than the overall Silin number of 27.72. One can see from Table 6 that lowering the H_K values by 8.9 will make the exponential terms in equation (18) reasonable but it will not lower the H values to a reasonable level.

By using the stalk Silin number to calculate a new H_K, equation (12) can be used to calculate C_n when C_o is known. As can be seen in Table 7, the predicted C_n were close to the actual C_n values. However, that Silin's mathematical treatment worked in this case should be taken with a grain of salt for several reasons. First, to get this model to work, only stalks were considered which only make up 20.9

Table 5. Calculation of the Overall Silin Number for Ensiled Chopped Sweet Sorghum

Section	Weight Percent	Silin Number (L) [m/100 g of sorghum]
Fine Mesh	33.0	45.99 ± 10.00
Little Leaves	23.0	25.90 ± 6.24
Little Stalks	7.6	5.25 ± 1.35
Medium Stalks	4.6	3.57 ± 0.46
Large Stalks	8.7	1.04 ± 0.06
Large Leaves	19.0	29.93 ± 10.02
Seed Heads	4.1	6.02 ± 1.41

The overall Silin number is determined by:

$$L \text{ (m/100 g sorghum)} = \Sigma \text{ (weight percent)}_i \, L_i$$

$$L = 27.72 \pm 6.82 \text{ m/100 g}$$

Table 6. Calculation of H Used in Equation 18

Temp. °C	L/S Ratio	n $\frac{\text{g Juice}}{\text{g Juice Sorghum}}$	Z $\frac{\text{g Juice Sorghum}}{\text{g Wet Sorghum}}$	Density of Juice in Sorghum θ_j [g/cm^3]	K	H	H_K
50	5.0	5.90	0.732	1.043	1.9×10^{-4}	3127	33.80
	4.5	5.25	0.732	1.043			
	4.0	4.35	0.732	1.043			
	3.5	3.54	0.756	1.042	1.8×10^{-4}	3025	31.99
	2.5	2.65	0.756	1.042			
70	5.0	6.33	0.686	1.044	2.0×10^{-4}	4453	50.81
	4.0	4.61	0.686	1.044			
	3.0	2.86	0.686	1.044			
	2.0	1.82	0.686	1.044			
	1.5	0.71	0.747	1.039	1.9×10^{-4}	4070	48.04

K, H and H_K are defined as follows:

$$K = 2.302 \frac{0.93}{Z} K_{Silin} = 2.302 \frac{0.93}{Z} 6.5 \times 10^{-5}$$

$$H = 400 \pi (L/Z) D t \rho_j$$

$$H_K = K \theta L t \rho_j$$

where t = diffusion time = 105 mins

L = 27.72 m/100 g sorghum

Equation 18:

$$C_n = \frac{C_o (n - 1)}{n \exp[((n - 1)/n) H] - 1}$$

At 50°C D is defined as (60):

$$D = 1 \times 10^{-6} \frac{60 \text{ s}}{1 \text{ min}} = 0.0006 \text{ cm}^2/\text{min}$$

and θ_{50} as (56):

$\theta_{50} = 58.6$ °K cm s/g

At 70°C D is defined as (56):

D = 0.0008 cm^2/min

and θ_{70} as:

$\theta_{70} = 83.6$ °K cm s/g

Table 7. Calculated C_n From Equation 18 Using the Average Silin Number for Stalks

Temp. °C	L/S Ratio	n $\frac{\text{g Juice}}{\text{g Juice Sorghum}}$	H_K Stalks	C_o $\frac{\text{g Juice Sorghum}}{\text{g Wet Sorghum}}$	C_n $\frac{\text{mg Sugar}}{\text{g Juice Sorghum}}$	Predicted C_n
50	5.0	5.90	3.80	59.7	2.49	2.13
	4.5	5.25			3.15	2.25
	4.0	4.35			3.34	2.49
	3.5	3.54	3.60	64.5	1.54	3.57
	2.5	2.65			3.97	4.45
70	5.0	6.33	5.72	63.3	1.12	0.43
	4.0	4.61			2.93	0.56
	3.0	2.86			1.77	1.01
	2.0	1.82			3.09	2.26
	1.5	0.71	5.41	48.8	3.07	15.30

$$C_n = \frac{C_o (n - 1)}{n \exp[((n - 1)/n) H_K] - 1}$$

percent of the weight of the ensiled chopped sweet sorghum. The fine mesh section which probably contains a good percentage of the sugars, and is 33 percent of the weight of the chopped ensilage was neglected. Secondly, the value of K was influenced by the material being extracted. The value of K_{Silin} used to calculate the K used for this work was for sugar beets and not sweet sorghum. Thirdly, Bruniche-Olsen (2,40) found Fick's first law to be invalid for sugar beets thinner than 4 mm. The stalks and the seed heads were thicker than 4 mm, but the leaves and fine mesh were not. This helps explain why the exponential term in equation (18) was unbounded or too large when the overall Silin number was used. Finally, was the summary treatment of the gradient of concentration. More complicated models which make allowance for the change in juice concentration within the sorghum during the extraction process, such as the one by Bruniche-Olsen (2), should be investigated.

2. Fermentation Studies

After extracting the ensiled sweet sorghum, the diffusion juice was concentrated to a suitable level and then fermented by Saccharomyces uvarum (Fermentation 1), Clostridium acetobutylicum (Fermentation 2, 3, and 6), and Zymomonas mobilis (Fermentation 4 and 5).

Figures 25 A and B show the time course for the S. uvarum fermentation performed on the 70°C juice extract. As the figures show, glucose and fructose were the substrates utilized while ethanol and lactic acid were the major products. Acetic acid and propionic acid were also formed but in minor amounts. The formation of lactic acid suggested contamination. Looking at the plots, there was a jump

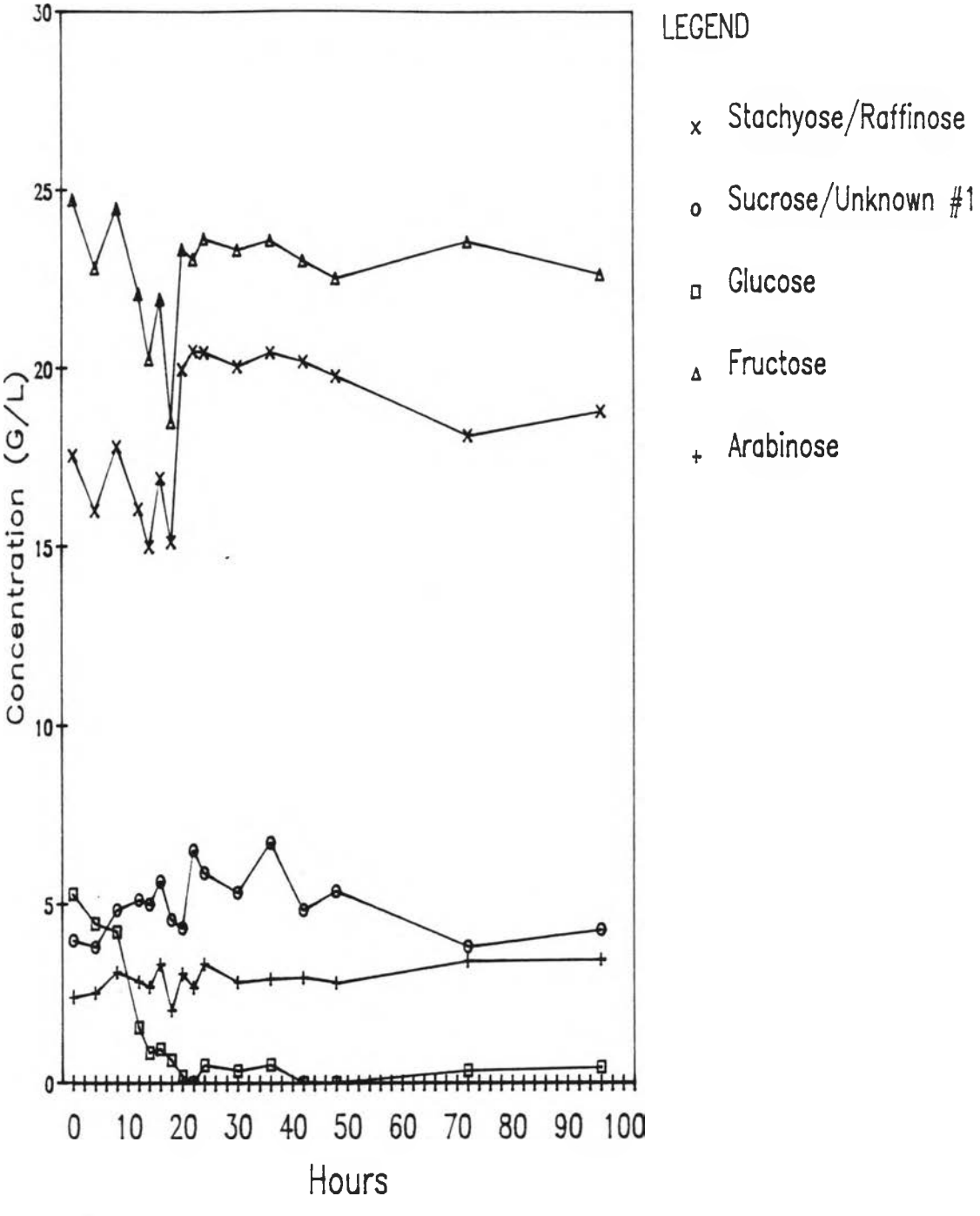


Figure 25A. Saccharomyces uvarum fermentation on 70°C juice extract. A. Substrates.

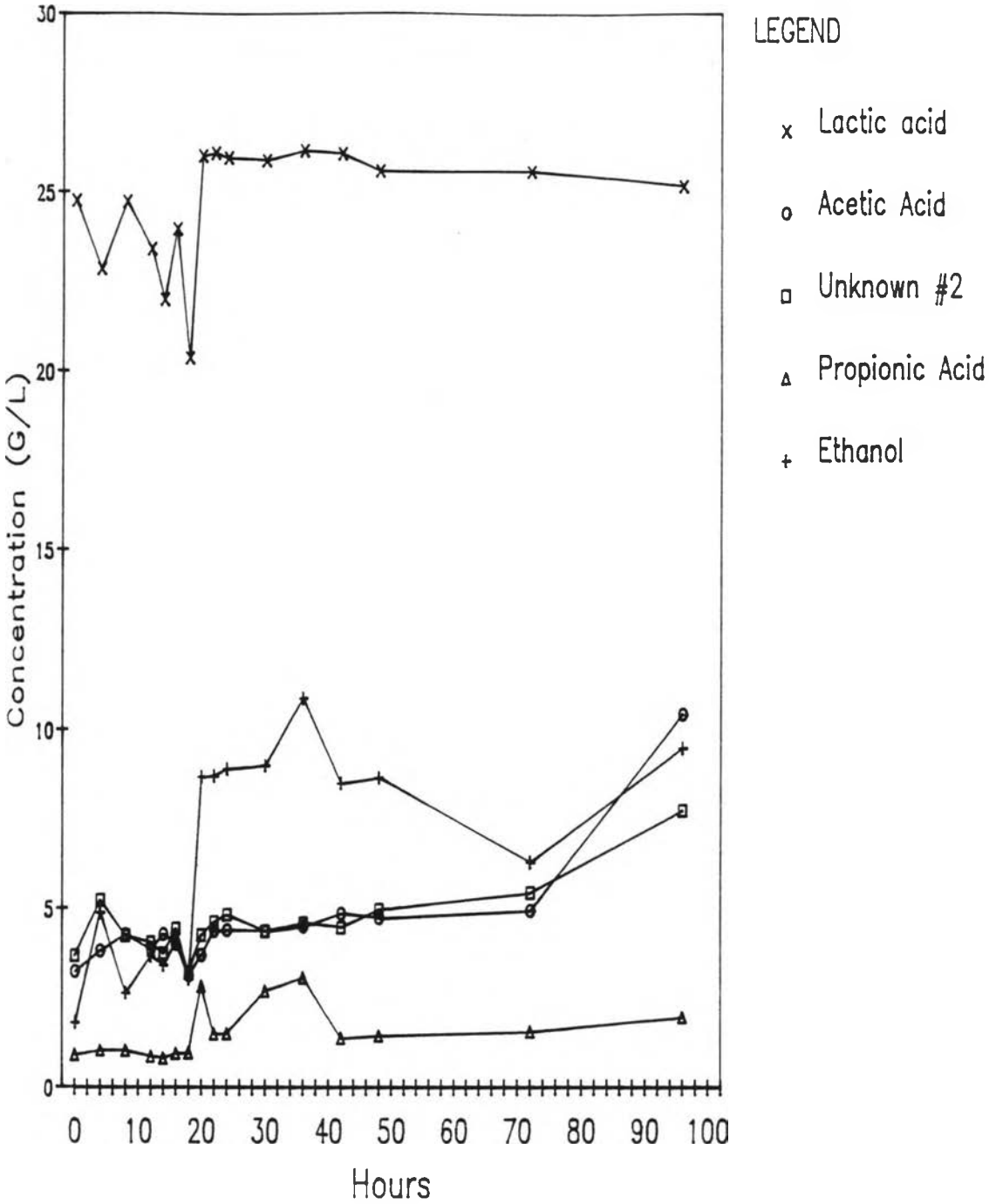


Figure 25B. Saccharomyces uvarum fermentation on 70°C juice extract. B. Products.

in concentrations at the 20 hour mark. This was where a recalibration of the standard was done, which increased the response factor thereby increasing concentration values. The other point was that stachyose was formed. Glucose and fructose were used immediately and acetic acid was formed immediately, but ethanol, lactic acid, and propionic acid production did not start until between 18 and 20 hours into the fermentation. It was also interesting to note that up to the 20 hour mark, it looked like stachyose and lactic acid were being consumed. This fermentation had a yield coefficient on ethanol (ethanol produced over substrates consumed) of 0.86 and a final ethanol concentration of 8.8 g/L. S. uvarum used 18 percent of the available substrates not including stachyose and lactic acid.

Figures 26 A and B show the time course for the C. acetobutylicum fermentation on the 70°C juice extract. Glucose, lactic acid, sucrose, arabinose, fructose and other pentoses, were all utilized between 24 to 40 hours; glucose 0 to 30 hours, lactic acid 24 to 40 hours, sucrose 32 to 42 hours, arabinose 28 to 36 hours, and fructose 30 to 36 hours. Oligosaccharides, quantified as stachyose, were consumed after 40 hours.

Figure 26B shows the time course of products formation. Butyric acid was produced as the substrates were utilized (24 hours) and leveled off at about 36 hours, the end of substrate consumption. Butanol production started and leveled off a little later, 30 to 40 hours respectively, in the same time frame as substrate utilization. Acetate was also produced within the time frame of substrate utilization, 28 to 60 hours. Slight increases in product concentration after 40 hours were due to oligosaccharide consumption. Increases in

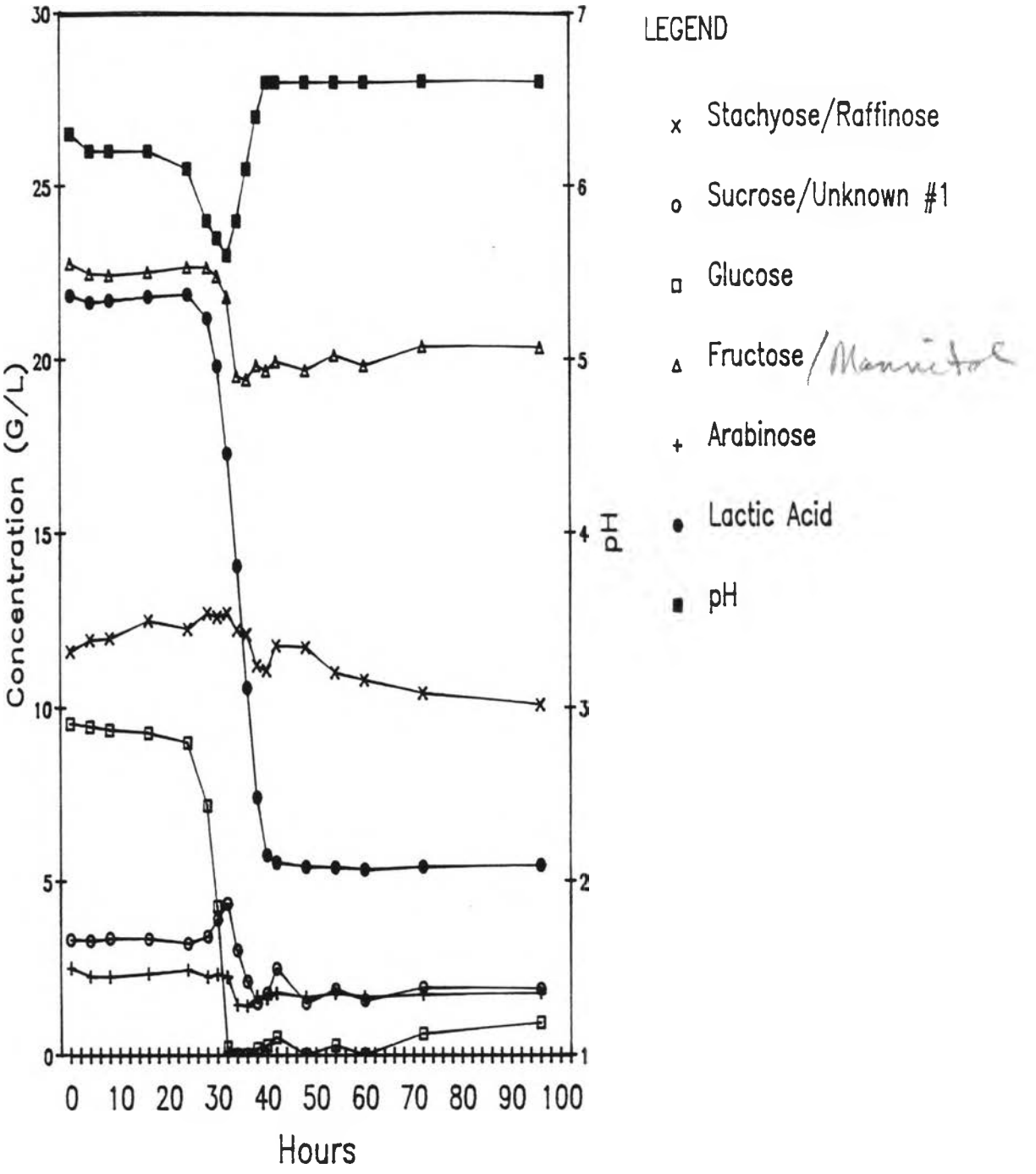


Figure 26A. Clostridium acetobutylicum fermentation on 70°C juice extract. A. Substrates and pH.

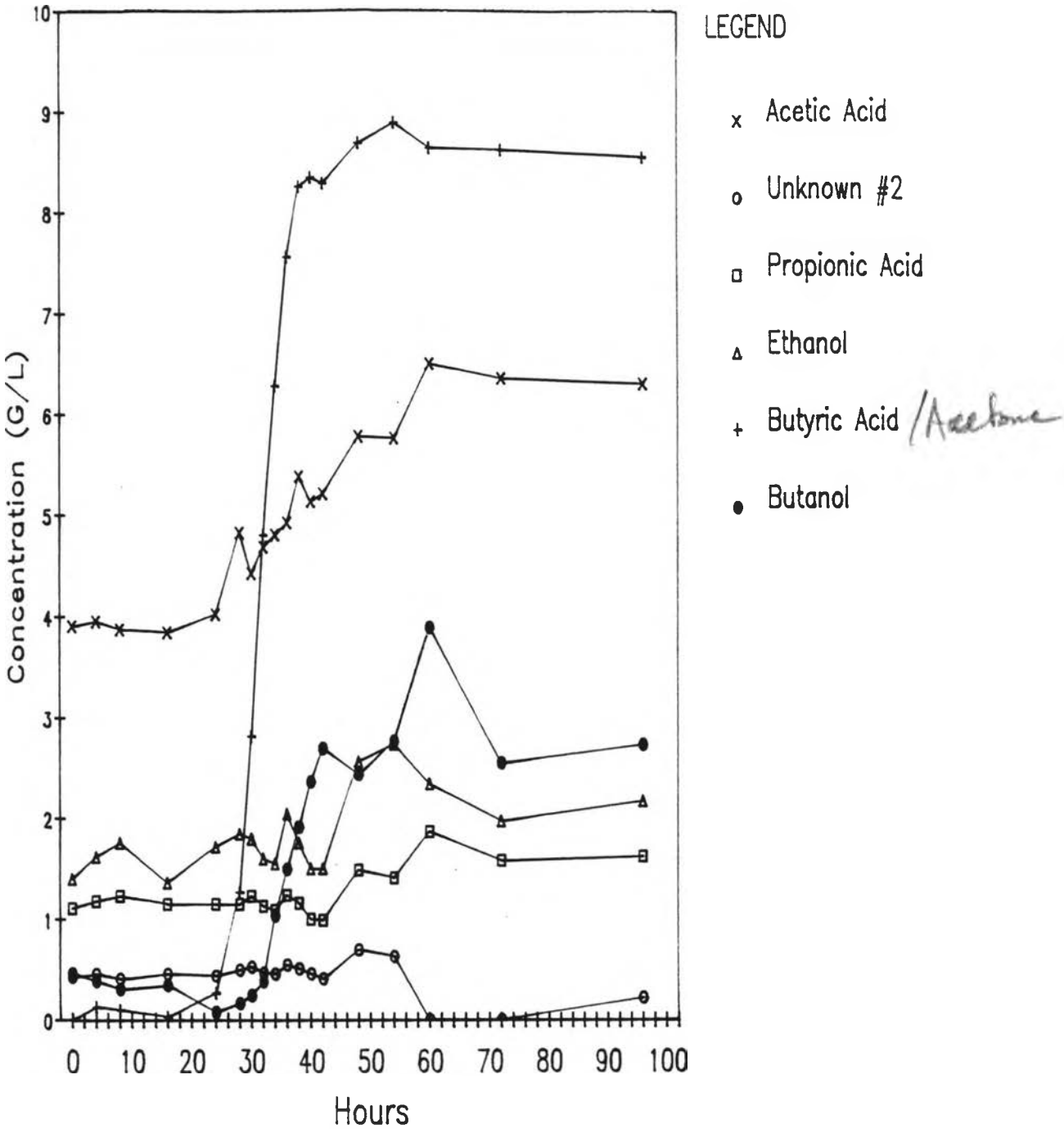


Figure 26B. Clostridium acetobutylicum fermentation on 70°C juice extract. B. Products.

propionic acid and ethanol were also due to oligosaccharide consumption since production started after 40 hours.

For this particular fermentation the yield coefficient (total acids and solvents produced over the substrates consumed) was 0.46 and 44 percent of the available substrates, including stachoyse and lactic acid were utilized. The problem was that high levels of butyric acid (8.7 g/L) instead of butanol (2.6 g/L) were formed, because the fermentation never seemed to reach the acid break point typical of C. acetobutylicum fermentations. The pH was not controlled. As Figure 26A shows the pH started at 6.3, dropped to 5.6 at 32 hours and climbed back to pH 6.6 at 40 hours. These transititions correspond with the start and stop of solvent production. The pH never dropped to 5, the pH at which the acid break point usually occurs.

A very important point was that C. acetobutylicum was capable of utilizing lactic acid, a by-product of the ensiling, as a substrate. This was important because lactic acid represents 30 percent of the substrate. Utilization would make ensiling a productive preprocessing step to fermentation.

Figures 27 A and B show the time course for the C. acetobutylicum fermentation on the 50^{o}C juice extract. Glucose, fructose, and lactic acid were the substrates utilized; glucose 0 to 24 hours, fructose 0 to 28 hours, and lactic acid 0 to 26 hours.

Figure 27B shows the time course of products formation. The minor products formed, acetic acid and ethanol, coincided with substrate utilization, 0 to 24 hours and 0 to 26 hours respectively. Butyric acid also coincided with substrate utilization, 0 to 24 hours. The last three points on the curve for each component were probably due to

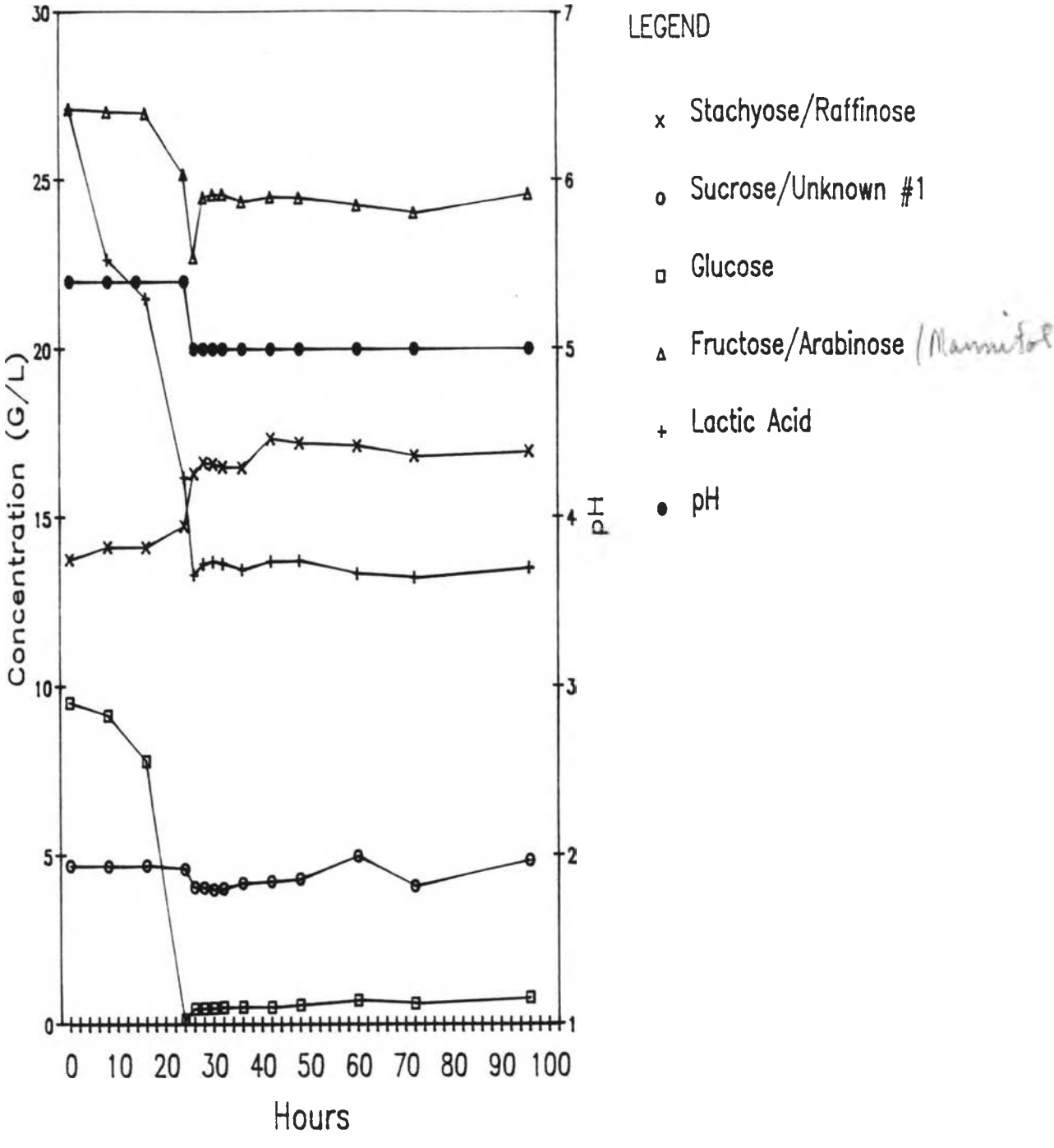


Figure 27A. Clostridium acetobutylicum fermentation on 50°C juice extract. A. Substrates and pH.

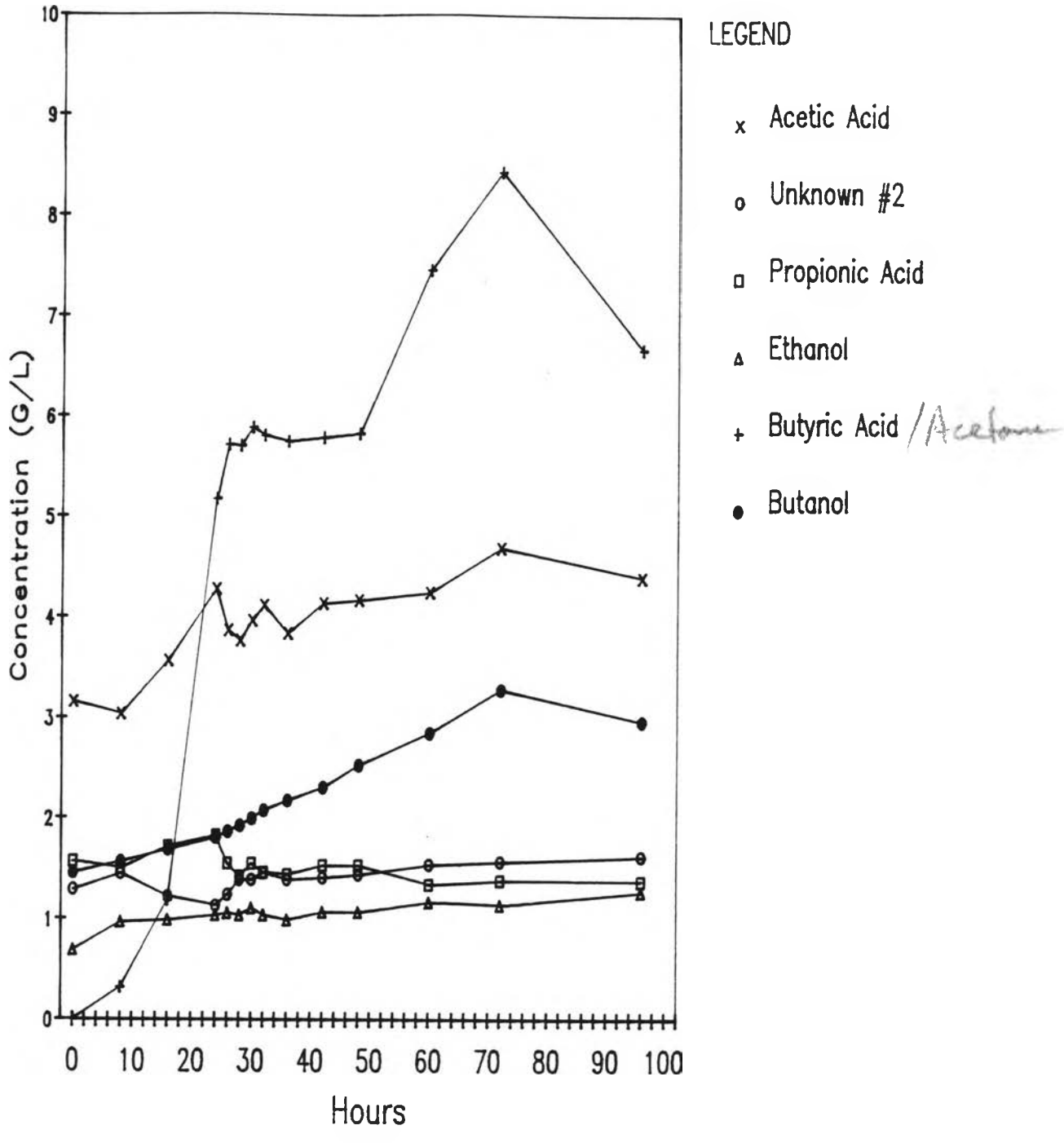


Figure 27B. Clostridium acetobutylicum fermentation on 50°C juice extract. B. Products.

HPLC error because those samples were analyzed with a new standard, which would yield a new response factor and thus different concentrations. Butanol was formed throughout the fermentation, 0 to 72 hours. Also in this fermentation some oligosaccharides were formed.

For this fermentation the yield coefficient (total acids and solvents produced over the substrates consumed) was 0.43, very similar to the C. acetobutylicum fermentation on the 70°C juice extract (0.46). However, for this fermentation only 31 percent of the available substrates, including stachyose and lactic, were utilized.

It was interesting to note that approximately the same amount of butyric acid (7.5 g/L) and butanol (3.0 g/L) were formed for this fermentation compared to the last one because the pH was controlled for this fermentation. The initial pH was 5.4, forced down to 5 at 25 hours, and was controlled at pH 5 for the duration of the fermentation (fig. 27A). This was the pH at which the acid break point should have occured, calling for more butanol to be formed. The reason the acid break point did not occur was unknown.

Between S. uvarum and C. acetobutylicum the organism best suited for fermentation of the ensiled sweet sorghum was the C. acetobutylicum. Even though S. uvarum had a higher yield it used less of the available substrates in the sweet sorghum. The advantage of C. acetobutylicum was that it utilized lactic acid, the product of the Lactobacillus fermentation which served to preserve the remainder of the fermentable sugars, and one of the most abundant materials in the ensiled sweet sorghum.

Three more fermentations were attempted, two with Zymomonas mobilis and one with C. acetobutylicum. The additional C. acetobutylicum

fermentation was attempted to achieve the acid break. None of the fermentations worked and tables of the component concentrations against time can be seen in Appendix A. There were two reasons why the Z. mobilis fermentations did not work. One could be because Z. mobilis was inhibited by lactic acid, and the other could be the sorghum juice was in the freezer to long and degraded the juice. This was probably the reason since the C. acetobutylicum fermentations did not work, but had worked initially.

Based on yields, it could be said that no inhibitory or other detrimental substances were formed during the ensiling or extraction steps. The fermentations had high yields and with the exception of S. uvarum, utilized a high percentage of the available substrates. Optimization of fermentation conditions could produce higher yields. On the other hand, the C. acetobutylicum fermentations never achieved the acid break point. Maybe a detrimental substance stopped the acid break point and inhibited the production of the secondary metabolite, butanol.

It was hard to compare the ensiling-extraction-fermentation process to the solid-state fermentation (SSF) process since only one yeast fermentation was performed in this study. Bryan and Parrish (6) got yields of 80 percent for SSF and 73 percent for a conventional submerged fermentation. Kargi et al. (26) by optimizing the SSF fermentation got final ethanol concentrations in the juice (juice pressed from the solids) of 7.6 percent and only 2.2 percent final ethanol concentration from fermenting juice. Gibbons et al. (20) had an ethanol concentration in the fermented pulp exiting the semicontinuous SSF device of 6 percent (v/v) which corresponded to an

ethanol yield of 179 liters/10^3 kg of dry sweet sorghum. In this study, S. uvarum fermentation of ensiled sweet sorghum juice had a yield of 86 percent which corresponded to a concentration of 9.5 percent (v/v) ethanol. The SSF were all conducted using fresh sweet sorghum. Recall, sweet sorghum must be preserved for year long availability of the sugars, and ensiling is one of the best methods to store this crop. Therefore, studies should be conducted to determine the effects of ensiling on SSF of sweet sorghum.

CONCLUSIONS

1. Ensiling preserved the fermentable sugars in sweet sorghum, variety Rio, for 10 months, at which time the ensilage was used for extraction studies.
2. In general, as the L/S ratio decreased, the pulp component concentrations increased. This was due to a diminished concentration gradient between liquid and solid phases at the lower L/S ratios.
3. In general, as the L/S ratio decreased, the diffusion juice component concentrations increased. This was mainly due to the dilution effect at higher L/S ratios.
4. The highest concentration of total soluble components obtained was around 53 g/L at the 1.5 L/S ratio in the 70^{o}C study. The concentration of fermentable sugars for the same run was approximately 3 percent (w/v). Therefore, concentrations of fermentable sugars in the diffusion juice suitable for fermentation (6 % w/v) were not obtainable from this particular diffuser.
5. In general, the diffuser had extraction efficiencies on pulp greater than 90 percent for all L/S ratios at both diffusion temperatures. Even though 90 percent of the components were removed from the sweet sorghum ensilage, less than 90 percent of the components were found in the outlet diffusion juice. The extraction efficiency based on juice was unsteady and in most cases less than 90 percent.

6. Based on data from run 10, the diffuser had an unsteady state nature. This data was collected for 7 hours at the 70°C 1.5 L/S ratio. The juice component concentrations increased with time. If the diffuser was at steady state the juice component concentrations would have remained level with time.

7. Silin's theoretical treatment of diffusion could predict the pulp component concentrations (equation 18), but only when using a stalk Silin number and the value of K from Silin of 6.5×10^{-5}. However, this model made several simplifying assumptions that do not apply to this particular system. The first assumptions of steady state and the concentration gradient equal to the difference in concentration between the sorghum and the diffusion juice over simplify the diffusion process. Secondly, the stalk Silin number was not a true representative Silin number for the chopped ensiled sweet sorghum used in these studies. Finally, the K value used was obtained from sugar beets.

8. The Saccharomyces uvarum fermentation on concentrated extraction juice had a yield coefficient on ethanol of 0.86 and a final ethanol concentration of 8.8 g/L. S. uvarum only used 18 percent of the available substrates, mostly glucose and fructose.

9. The Clostridium acetobutylicum fermentation on concentrated extraction juice with no pH control had a yield coefficient of 0.46 and consumed 44 percent of the available substrates. The C. acetobutylicum fermentation with pH control had a yield coefficient of 0.43 and utilized 31 percent of the available substrates. For both these fermentations the acid break point did not occur, meaning low levels of butanol were formed, 2.6 g/L for the fermentation with pH control, and

3.0 g/L for the fermentation without pH control. The reason the acid break point did not occur was unknown.

10. Between S. uvarum and C. acetobutylicum, the organism best suited for fermentation of the ensiled sweet sorghum diffusion juice was C. acetobutylicum. This was because C. acetobutylicum was capable of metabolizing lactic acid, a major by-product of ensiling.

11. It was inconclusive as to whether or not inhibitory or other detrimental substances were formed during the ensiling or extraction steps.

12. No legitimate comparision can be made between the ensiling-extraction-fermentation process and solid substrate fermentation until solid substrate fermentation is studied on ensiled sweet sorghum.

RECOMMENDATIONS

1. The polyethylene bags used to create silos should be washed prior to use to help identify unknown #1 as a component of the sweet sorghum or as a plasticyin.

2. Future extraction studies should establish the time to reach equilibrium for specific independent factors (i.e., liquid-solid ratio and temperature).

3. Theoretical treatments of continuous countercurrent diffusion which are more representative of the extraction conditions and ensilage used in this study should be investigated. These should acknowledge rigorous treatment of several parameters, including concentration gradients, ensilage shape, and the steady state assumption.

4. The Clostridium acetobutylicum fermentations of the ensiled sweet sorghum diffusion juice should be further studied . This study should focus on optimization of the fermentation and in determining why the acid break point was not reached.

5. Solid substrate fermentation of ensiled sweet sorghum should be thoroughly studied to determine the effects of ensiling on the overall yield of ethanol.

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APPENDIX A

Extraction Data and Sample Calculations

High Performace Liquid Chromatography (HPLC) Analysis of
Sweet Sorghum Ensilage (mg/ml) Prepared by the Blender Extraction Method*

Component	FE 8/20	FE 8/21	FE 8/22	FE 8/23
Stachyose/ Raffinose	0.49 ± 0.03	0.47 ± 0.04	0.27 ± 0.02	0.33 ± 0.03
Sucrose/ Unknown #1	0.45 ± 0.02	0.41 ± 0.06	0.42 ± 0.03	0.39 ± 0.04
Glucose	0.76 ± 0.08	0.48 ± 0.05	0.73 ± 0.02	0.38 ± 0.02
Fructose/ Arabinose	3.17 ± 0.32	2.82 ± 0.31	2.80 ± 0.02	3.08 ± 0.01
Lactic Acid	2.63 ± 0.23	2.28 ± 0.23	2.50 ± 0.12	2.54 ± 0.03
Acetic Acid	0.70 ± 0.05	0.68 ± 0.10	0.69 ± 0.12	0.78 ± 0.14
Unknown #2	0.07 ± 0.06	0.08 ± 0.03	0.01 ± 0.01	0.04 ± 0.03
Propionic Acid	0.16 ± 0.06	0.20 ± 0.05	0.12 ± 0.06	0.10 ± 0.01
Ethanol	0.86 ± 0.12	0.83 ± 0.13	0.78 ± 0.04	1.00 ± 0.09
Total	9.29 ± 0.87	8.25 ± 0.99	8.32 ± 0.31	8.66 ± 0.01
Sugars	4.87 ± 0.35	4.18 ± 0.46	4.22 ± 0.04	4.18 ± 0.04
Organic Acids	4.42 ± 0.52	4.07 ± 0.53	4.10 ± 0.28	4.46 ± 0.04

* Average of 3 HPLC Analysis.

High Performance Liquid Chromatography (HPLC) Analysis of Sweet Sorghum Ensilage (mg/g Dry Weight) Prepared by the Blender Extraction Method*

Component	FE 8/20	FE 8/21	FE 8/22	FE 8/23
Stachyose/ Raffinose	15.50 ± 0.95	16.78 ± 1.43	7.26 ± 0.54	11.19 ± 1.02
Sucrose/ Unknown #1	14.24 ± 0.63	14.64 ± 2.14	11.28 ± 0.81	13.22 ± 1.36
Glucose	24.05 ± 2.53	17.14 ± 1.78	19.62 ± 0.54	12.88 ± 0.68
Fructose/ Arabinose	100.31 ± 10.12	100.71 ± 11.07	75.24 ± 0.54	104.41 ± 0.34
Lactic Acid	83.22 ± 7.28	81.42 ± 8.21	67.18 ± 3.22	86.10 ± 1.02
Acetic Acid	22.15 ± 1.58	24.28 ± 3.57	18.54 ± 3.22	26.44 ± 4.74
Unknown #2	2.21 ± 1.90	2.86 ± 1.07	0.27 ± 0.27	1.36 ± 1.02
Propionic Acid	5.06 ± 1.90	7.14 ± 1.78	3.22 ± 1.61	3.39 ± 0.34
Ethanol	27.21 ± 3.80	29.64 ± 4.64	20.96 ± 1.07	33.90 ± 3.05
Total	293.95 ± 27.53	294.61 ± 35.36	223.57 ± 8.33	292.89 ± 0.34
Sugars	154.10 ± 11.07	149.27 ± 16.43	113.40 ± 1.07	141.70 ± 1.36
Organic Acids	139.85 ± 16.45	145.34 ± 18.93	110.17 ± 7.52	151.19 ± 1.36

* Average of 3 HPLC Analysis.

High Performance Liquid Chromatography (HPLC) Analysis of
Sweet Sorghum Ensilage (mg/ml) Prepared by the Expression Method*

Sample	Stachyose/ Raffinose	Sucrose/ Unknown #1	Glucose	Fructose/ Arabinose	Lactic Acid	Acetic Acid
FE 8/20						
#1	11.61	3.34	8.72	34.70	28.76	7.34
#2	12.46	4.80	8.96	36.43	29.90	7.42
#3	12.99	6.19	9.93	36.46	30.30	7.13
	12.35 ± 0.70	4.78 ± 1.42	9.20 ± 0.64	35.86 ± 1.01	29.65 ± 0.80	7.30 ± 0.15
FE 8/21						
#1	13.68	5.62	7.67	38.60	29.18	8.35
#2	14.00	4.82	8.11	41.78	31.70	8.88
	13.84 ± 0.23	5.22 ± 0.56	7.89 ± 0.31	40.19 ± 2.25	30.44 ± 1.78	8.62 ± 0.37
FE 8/22						
#1	11.61	6.16	10.28	37.50	32.40	8.10
#2	11.98	6.22	10.22	37.83	32.61	8.17
#3	12.04	6.14	10.12	37.62	32.21	8.21
	11.88 ± 0.23	6.17 ± 0.04	10.21 ± 0.08	37.65 ± 0.17	32.41 ± 0.20	8.16 ± 0.06
FE 8/23						
#1	10.02	2.67	4.30	36.33	29.58	7.93
#2	10.78	2.60	4.12	36.34	29.23	7.72
#3	7.91	2.47	3.67	31.50	25.50	6.75
	9.57 ± 1.49	2.58 ± 0.10	4.03 ± 0.32	34.72 ± 2.79	28.10 ± 2.26	7.47 ± 0.63

Sample	Unknown #2	Propionic Acid	Ethanol	Total	Sugars	Organic Acids
FE 8/20						
#1	1.16	2.25	9.26	107.14	58.37	48.77
#2	1.33	2.12	9.27	112.69	62.65	50.04
#3	1.23	2.00	8.94	115.17	65.57	49.60
	1.24 ± 0.08	2.12 ± 0.12	9.16 ± 0.19	111.66 ± 4.11	62.19 ± 3.62	49.47 ± 0.64
FE 8/21						
#1	1.30	2.16	9.84	116.40	65.57	50.83
#2	1.46	2.52	10.07	123.34	68.71	54.63
	1.38 ± 0.11	2.34 ± 0.25	9.96 ± 0.16	119.88 ± 4.91	67.14 ± 2.22	52.74 ± 2.69
FE 8/22						
#1	0.50	1.17	9.10	116.82	65.55	51.27
#2	1.30	1.61	9.72	119.66	66.25	53.41
#3	0.46	1.20	8.09	116.09	65.92	50.17
	0.75 ± 0.47	1.33 ± 0.24	8.97 ± 0.82	117.53 ± 1.89	65.91 ± 0.35	51.62 ± 1.65
FE 8/23						
#1	0.83	1.53	10.83	104.02	53.32	50.70
#2	0.46	1.38	10.61	103.24	53.84	49.40
#3	0.42	1.24	8.42	87.88	45.55	42.33
	0.57 ± 0.23	1.38 ± 0.14	9.95 ± 1.33	98.37 ± 9.10	50.90 ± 4.64	47.47 ± 4.50

* Average of 2 HPLC Anaylsis.

Averages of Sweet Sorghum Ensilage Composition (mg/g Dry Weight)
Prepared by the Expression Method

Component	FE 8/20	FE 8/21	FE 8/22	FE 8/23
Stachyose/ Raffinose	32.36 ± 1.83	41.24 ± 0.68	24.83 ± 0.48	27.18 ± 4.23
Sucrose/ Unknown #1	12.52 ± 3.72	15.56 ± 1.67	12.90 ± 0.08	7.33 ± 0.28
Glucose	24.10 ± 1.68	23.51 ± 0.92	21.34 ± 0.17	11.44 ± 0.91
Fructose/ Arabinose	93.95 ± 2.65	119.77 ± 6.70	78.69 ± 0.36	98.60 ± 7.92
Lactic Acid	77.68 ± 2.10	90.71 ± 5.30	67.74 ± 0.42	79.80 ± 6.42
Acetic Acid	19.13 ± 0.39	25.69 ± 1.10	17.05 ± 0.12	21.21 ± 1.79
Unknown #2	3.25 ± 0.21	4.11 ± 0.33	1.57 ± 0.98	1.62 ± 0.65
Propionic Acid	5.55 ± 0.31	6.97 ± 0.74	2.78 ± 0.50	3.92 ± 0.40
Ethanol	24.00 ± 0.50	29.68 ± 0.48	18.75 ± 1.71	28.26 ± 3.78
Total	292.54 ± 10.77	357.24 ± 14.63	245.65 ± 3.95	279.36 ± 25.84
Sugars	162.93 ± 9.48	200.08 ± 6.62	137.76 ± 0.73	144.55 ± 13.18
Organic Acids	129.61 ± 1.68	157.16 ± 8.02	107.89 ± 3.45	134.81 ± 12.78

High Performance Liquid Chromatography (HPLC) Analysis of Pulp Samples (mg/g) for the 50°C Runs Prepared by the Expression Method*

Sample	Stachyose/ Raffinose	Sucrose/ Unknown #1	Glucose	Fructose/ Arabinose	Lactic Acid	Acetic Acid
P 5.0/1						
#1	0.73	0.13	0.29	1.12	0.75	0.00
#2	0.78	0.12	0.33	1.30	1.04	0.11
#3	0.91	0.18	0.31	1.28	0.95	0.08
	0.81 ± 0.09	0.14 ± 0.03	0.31 ± 0.02	1.23 ± 0.10	0.91 ± 0.15	0.06 ± 0.06
P 4.5/1						
#1	0.58	0.19	0.65	2.04	1.55	0.13
#2	0.53	0.22	0.50	1.70	1.25	0.14
#3	0.74	0.17	0.48	1.65	0.13	0.18
	0.62 ± 0.11	0.19 ± 0.02	0.54 ± 0.09	1.80 ± 0.21	1.31 ± 0.22	0.15 ± 0.03
P 4.0/1						
#1	0.67	0.33	0.72	2.18	1.35	0.15
#2	0.48	0.15	0.47	1.60	0.99	0.10
#3	0.69	0.24	0.56	1.95	1.18	0.10
	0.61 ± 0.12	0.24 ± 0.09	0.58 ± 0.13	1.91 ± 0.29	1.17 ± 0.18	0.12 ± 0.03
P 3.5/1						
#1	0.15	0.10	0.15	0.72	0.33	0.0
#2	0.30	0.12	0.27	1.27	0.75	0.12
#3	0.19	0.12	0.21	1.03	0.67	0.08
	0.21 ± 0.18	0.11 ± 0.01	0.21 ± 0.06	1.01 ± 0.28	0.58 ± 0.22	0.07 ± 0.06
P 2.5/1						
#1	0.75	0.31	0.47	2.40	1.61	0.25
#2	0.82	0.32	0.50	2.61	1.79	0.25
#3	1.01	0.21	0.37	2.15	1.52	0.26
	0.86 ± 0.13	0.28 ± 0.06	0.45 ± 0.07	2.39 ± 0.23	1.64 ± 0.14	0.25 ± 0.00

Sample	Unknown #2	Propionic Acid	Ethanol	Total	Sugars	Organic Acid
P 5.0/1						
#1	0.00	0.00	0.00	3.02	2.27	0.75
#2	0.00	0.00	0.00	3.68	2.53	1.15
#3	0.00	0.00	0.00	3.71	2.68	1.03
	0.00 ± 0.00	0.00 ± 0.00	0.00 ± 0.00	3.46 ± 0.39	2.49 ± 0.21	0.97 ± 0.20
P 4.5/1						
#1	0.00	0.00	0.00	5.14	3.46	1.68
#2	0.00	0.06	0.00	4.40	2.95	1.45
#3	0.00	0.00	0.00	4.35	3.04	1.31
	0.00 ± 0.00	0.02 ± 0.03	0.00 ± 0.00	4.63 ± 0.44	3.15 ± 0.27	1.48 ± 0.19
P 4.0/1						
#1	0.00	0.00	0.00	5.40	3.90	1.50
#2	0.00	0.00	0.00	3.79	2.70	1.09
#3	0.06	0.00	0.00	4.78	3.44	1.34
	0.02 ± 0.03	0.00 ± 0.00	0.00 ± 0.00	4.65 ± 0.81	3.34 ± 0.60	1.31 ± 0.21
P 3.5/1						
#1	0.00	0.00	0.00	1.45	1.12	0.33
#2	0.00	0.00	0.00	2.83	1.96	0.87
#3	0.00	0.00	0.00	2.30	1.55	0.75
	0.00 ± 0.00	0.00 ± 0.00	0.00 ± 0.00	2.19 ± 0.70	1.54 ± 0.42	0.65 ± 0.28
P 2.5/1						
#1	0.03	0.00	0.15	5.97	3.93	2.04
#2	0.00	0.07	0.22	6.58	4.25	2.33
#3	0.00	0.00	0.23	5.75	3.74	2.01
	0.01 ± 0.02	0.02 ± 0.04	0.20 ± 0.04	6.10 ± 0.43	3.98 ± 0.26	2.12 ± 0.18

* Originally, the analysis was performed with samples having units of mg/ml, but the density was 1 g/ml for all samples; therefore, the units for the samples are mg/g or mg/ml.

High Performance Liquid Chromatography (HPLC) Analysis of
Pulp Samples (Dry Weight mg/g) for the 50°C Runs Prepared by the Expression Method*

L/S Ratio	Stachyose/ Raffinose	Sucrose/ Unknown #1	Glucose	Fructose/ Arabinose	Lactic Acid	Acetic Acid
5.0	3.95 ± 0.44	0.68 ± 0.15	1.51 ± 0.10	6.00 ± 0.49	4.44 ± 0.73	0.29 ± 0.29
4.5	3.01 ± 0.53	0.92 ± 0.10	2.62 ± 0.44	8.73 ± 1.02	6.35 ± 1.07	0.73 ± 0.14
4.0	2.84 ± 0.56	1.12 ± 0.42	2.70 ± 0.60	8.88 ± 1.35	5.44 ± 0.84	0.56 ± 0.14
3.5	1.01 ± 0.38	0.53 ± 0.05	1.01 ± 0.29	4.86 ± 1.35	2.79 ± 1.06	0.34 ± 0.29
2.5	4.69 ± 0.71	1.53 ± 0.33	2.45 ± 0.38	13.02 ± 1.25	8.94 ± 0.76	1.36 ± 0.00

L/S Ratio	Unknown #2	Propionic Acid	Ethanol	Total	Sugars	Organic Acids
5.0	0.00 ± 0.00	0.00 ± 0.00	0.00 ± 0.00	16.87 ± 1.90	12.14 ± 1.02	4.73 ± 0.98
4.5	0.00 ± 0.00	0.10 ± 0.14	0.00 ± 0.00	22.46 ± 2.13	15.28 ± 1.31	7.18 ± 0.92
4.0	0.09 ± 0.14	0.00 ± 0.00	0.00 ± 0.00	21.63 ± 3.77	15.54 ± 2.79	6.09 ± 0.98
3.5	0.00 ± 0.00	0.00 ± 0.00	0.00 ± 0.00	10.54 ± 3.37	7.41 ± 2.02	3.13 ± 1.35
2.5	0.05 ± 0.11	0.11 ± 0.22	1.09 ± 0.22	33.24 ± 2.34	21.69 ± 1.42	11.55 ± 0.98

* Average of 3 samples.

High Performance Liquid Chromatography (HPLC) Analysis of
Pulp Samples (mg/g) for the 70°C Runs Prepared by the Expression Method*

Sample	Stachyose/ Raffinose	Sucrose/ Unknown #1	Glucose	Fructose/ Arabinose	Lactic Acid	Acetic Acid
P 5.0/1						
#1	0.12	0.08	0.19	0.78	0.26	0.00
#2	0.14	0.03	0.16	0.72	0.35	0.00
#3	0.15	0.03	0.19	0.76	0.34	0.00
	0.14 ± 0.02	0.05 ± 0.03	0.18 ± 0.02	0.75 ± 0.03	0.32 ± 0.05	0.00 ± 0.00
P 4.0/1						
#1	0.45	0.20	0.37	1.90	0.81	0.04
#2	0.40	0.27	0.50	1.93	0.85	0.06
#3	0.35	0.22	0.42	1.77	0.85	0.10
	0.40 ± 0.05	0.23 ± 0.04	0.43 ± 0.06	1.87 ± 0.08	0.84 ± 0.02	0.07 ± 0.03
P 3.0/1						
#1	0.21	0.12	0.32	1.30	0.65	0.00
#2	0.17	0.11	0.24	1.07	0.52	0.00
	0.19 ± 0.03	0.12 ± 0.01	0.28 ± 0.06	1.18 ± 0.16	0.58 ± 0.09	0.00 ± 0.00
P 2.0/1						
#1	0.36	0.22	0.56	2.02	1.26	0.08
#2	0.63	0.19	0.47	1.65	0.96	0.15
#3	0.45	0.26	0.54	1.95	1.22	0.12
	0.48 ± 0.14	0.22 ± 0.04	0.52 ± 0.05	1.87 ± 0.20	1.15 ± 0.16	0.12 ± 0.04
P 1.5A-E/1						
#1	0.52	0.17	0.43	1.77	1.32	0.16
#2	0.70	0.22	0.52	2.19	1.59	0.26
#3	0.47	0.22	0.41	1.62	1.32	0.22
	0.56 ± 0.12	0.20 ± 0.03	0.45 ± 0.06	1.86 ± 0.30	1.41 ± 0.16	0.21 ± 0.05
P 1.5F-J/1						
#1	1.23	0.52	0.97	4.20	3.41	0.68
#2	1.42	0.73	1.09	4.52	3.45	0.77
#3	1.36	0.57	1.00	4.63	3.59	0.80
	1.34 ± 0.10	0.61 ± 0.11	1.02 ± 0.06	4.45 ± 0.22	3.48 ± 0.09	0.75 ± 0.06

Sample	Unknown #2	Propionic Acid	Ethanol	Total	Sugars	Organic Acid
P 5.0/1						
#1	0.00	0.00	0.00	1.43	1.17	0.26
#2	0.00	0.00	0.00	1.40	1.05	0.35
#3	0.00	0.00	0.00	1.47	1.13	0.34
	0.00 ± 0.00	0.00 ± 0.00	0.00 ± 0.00	1.44 ± 0.04	1.12 ± 0.06	0.32 ± 0.05
P 4.0/1						
#1	0.00	0.00	0.00	3.77	2.92	0.85
#2	0.00	0.00	0.02	4.03	3.10	0.93
#3	0.00	0.00	0.00	3.71	2.76	0.95
	0.00 ± 0.00	0.00 ± 0.00	0.01 ± 0.01	3.85 ± 0.17	2.93 ± 0.17	0.92 ± 0.05
P 3.0/1						
#1	0.00	0.00	0.00	2.60	1.95	0.65
#2	0.00	0.00	0.00	2.11	1.59	0.52
	0.00 ± 0.00	0.00 ± 0.00	0.00 ± 0.00	2.35 ± 0.35	1.77 ± 0.25	0.58 ± 0.09
P 2.0/1						
#1	0.00	0.00	0.00	4.50	3.16	1.34
#2	0.00	0.00	0.00	4.05	2.94	1.11
#3	0.00	0.00	0.12	4.66	3.20	1.46
	0.00 ± 0.00	0.00 ± 0.00	0.04 ± 0.07	4.40 ± 0.32	3.09 ± 0.14	1.31 ± 0.18
P 1.5A-F/1						
#1	0.00	0.00	0.00	4.37	2.89	1.48
#2	0.00	0.00	0.00	5.48	3.63	1.85
#3	0.00	0.00	0.00	4.26	2.72	1.54
	0.00 ± 0.00	0.00 ± 0.00	0.00 ± 0.00	4.69 ± 0.67	3.07 ± 0.48	1.62 ± 0.20
P 1.5F-J/1						
#1	0.02	0.06	0.26	11.35	6.92	4.43
#2	0.00	0.00	0.21	12.19	7.76	4.43
#3	0.00	0.08	0.00	12.03	7.56	4.47
	0.01 ± 0.01	0.05 ± 0.04	0.16 ± 0.14	11.87 ± 0.45	7.42 ± 0.44	4.45 ± 0.02

* Originally, the analysis was performed with samples having units of mg/ml, but the density was 1 g/ml for all samples; therefore, the units for the samples are mg/g or mg/ml.

High Performance Liquid Chromatography (HPLC) Analysis of
Pulp Samples (Dry Weight mg/g) for the 70°C Runs Prepared by the Expression Method*

L/S Ratio	Stachyose/ Raffinose	Sucrose/ Unknown #1	Glucose	Fructose/ Arabinose	Lactic Acid	Acetic Acid
5.0	0.87 ± 0.12	0.31 ± 0.18	1.11 ± 0.12	4.64 ± 0.18	1.98 ± 0.31	0.00 ± 0.00
4.0	2.02 ± 0.25	1.16 ± 0.20	2.18 ± 0.30	9.46 ± 0.40	4.25 ± 0.10	0.35 ± 0.15
3.0	0.97 ± 0.15	0.61 ± 0.05	1.43 ± 0.31	6.02 ± 0.82	2.96 ± 0.46	0.00 ± 0.00
2.0	2.66 ± 0.78	1.22 ± 0.22	2.88 ± 0.28	10.36 ± 1.11	6.37 ± 0.89	0.66 ± 0.22
1.5	2.90 ± 0.62	1.03 ± 0.16	2.33 ± 0.31	9.62 ± 1.55	7.29 ± 0.83	1.08 ± 0.26
1.5F	6.87 ± 0.51	3.13 ± 0.56	5.23 ± 0.31	22.83 ± 1.13	17.85 ± 0.46	3.85 ± 0.31

L/S Ratio	Unknown #2	Propionic Acid	Ethanol	Total	Sugars	Organic Acids
5.0	0.00 ± 0.00	0.00 ± 0.00	0.00 ± 0.00	8.91 ± 0.25	6.93 ± 0.37	1.98 ± 0.31
4.0	0.00 ± 0.00	0.00 ± 0.00	0.05 ± 0.05	19.47 ± 0.86	14.82 ± 0.86	4.65 ± 0.25
3.0	0.00 ± 0.00	0.00 ± 0.00	0.00 ± 0.00	11.99 ± 1.78	9.03 ± 1.28	2.96 ± 0.46
2.0	0.00 ± 0.00	0.00 ± 0.00	0.22 ± 0.39	24.37 ± 1.77	17.12 ± 0.78	7.25 ± 1.00
1.5	0.00 ± 0.00	0.00 ± 0.00	0.00 ± 0.00	24.25 ± 3.46	15.88 ± 2.48	8.37 ± 1.03
1.5F	0.05 ± 0.05	0.26 ± 0.20	0.82 ± 0.72	60.89 ± 2.31	38.06 ± 2.26	22.83 ± 0.10

* Average of 3 samples.

High Performance Liquid Chromatography (HPLC) Analysis of Juice Samples (mg/ml) for the 50°C Runs*

L/S Ratio	Sample	Stachyose/ Raffinose	Sucrose/ Unknown #1	Glucose	Fructose/ Arabinose	Lactic Acid	Acetic Acid
5.0	1A	1.14	0.62	0.97	4.40	4.39	0.84
	B	1.00	0.62	1.19	4.64	4.46	0.36
	C						
	D	1.21	0.69	1.14	4.74	4.64	0.53
		1.12 ± 0.11	0.64 ± 0.04	1.10 ± 0.12	4.59 ± 0.17	4.50 ± 0.13	0.58 ± 0.24
4.5	2A	0.25	0.33	0.57	2.17	2.24	0.29
	B						
	C	1.48	1.01	1.77	6.26	5.76	1.30
	D	1.34	0.88	1.68	6.05	5.39	0.73
		1.02 ± 0.67	0.74 ± 0.36	1.34 ± 0.67	4.83 ± 2.30	4.46 ± 1.93	0.77 ± 0.51
4.0	3A	1.70	1.20	1.87	6.98	6.04	1.48
	B	2.13	1.46	2.30	8.54	7.44	1.87
	C	1.97	1.19	2.00	8.05	7.14	1.82
	D	1.99	1.03	1.97	8.30	7.23	1.83
		1.95 ± 0.18	1.22 ± 0.18	2.04 ± 0.18	7.97 ± 0.69	6.96 ± 0.63	1.75 ± 0.18
3.5	4A	1.20	0.54	0.87	5.36	4.86	1.10
	B	1.54	0.66	0.99	6.60	6.16	1.54
	C	1.99	1.00	1.31	7.49	6.81	1.66
	D	2.32	1.28	1.76	8.66	7.18	2.04
		1.76 ± 0.49	0.87 ± 0.34	1.23 ± 0.40	7.03 ± 1.40	6.25 ± 1.02	1.58 ± 0.39
2.5	5A	1.95	0.86	1.36	7.60	6.63	2.37
	B	2.56	1.27	1.79	9.24	7.90	2.14
	C	2.76	1.49	2.03	9.84	8.24	2.71
		2.42 ± 0.42	1.21 ± 0.32	1.73 ± 0.34	8.89 ± 1.16	7.59 ± 0.85	2.41 ± 0.29

L/S Ratio	Sample	Unknown #2	Propionic Acid	Ethanol	Total	Sugars	Organic Acid
5.0	1A	0.26	0.38	0.98	13.98	7.13	6.85
	B	0.19	0.30	0.47	13.23	7.45	5.78
	C	Lost Sample					
	D	0.24	0.34	0.74	14.27	7.78	6.49
		0.23 ± 0.04	0.34 ± 0.04	0.73 ± 0.26	13.83 ± 0.54	7.45 ± 0.33	6.38 ± 0.54
4.5	2A	0.05	0.23	0.35	6.48	3.32	3.16
	B	Lost Sample					
	C	0.26	0.42	1.54	19.80	10.52	9.28
	D	0.19	0.32	1.04	17.62	9.95	7.67
		0.17 ± 0.11	0.32 ± 0.10	0.98 ± 0.60	14.63 ± 7.14	7.93 ± 4.00	6.70 ± 3.17
4.0	3A	0.24	0.46	1.68	21.65	11.75	9.90
	B	0.32	0.54	2.02	26.62	14.43	12.19
	C	0.44	0.60	1.91	25.12	13.21	11.91
	D	0.33	0.56	1.95	25.19	13.29	11.90
		0.33 ± 0.08	0.54 ± 0.06	1.89 ± 0.15	24.65 ± 2.11	13.18 ± 1.10	11.47 ± 1.06
3.5	4A	0.12	0.26	1.19	15.50	7.97	7.53
	B	0.26	0.46	1.86	20.07	9.79	10.28
	C	0.29	0.52	1.78	22.85	11.79	11.06
	D	0.32	0.60	2.30	26.46	14.02	12.44
		0.25 ± 0.09	0.46 ± 0.14	1.78 ± 0.46	21.21 ± 4.62	10.89 ± 2.60	10.32 ± 2.07
2.5	5A	0.30	0.53	2.02	23.62	11.77	11.85
	B	0.35	0.65	2.32	28.22	14.86	13.36
	C	0.37	0.64	1.56	29.64	16.12	13.52
		0.34 ± 0.04	0.61 ± 0.07	1.97 ± 0.38	27.17 ± 3.15	14.25 ± 2.24	12.92 ± 0.92

* Average of 2 or 3 HPLC analysis readings.

High Performance Liquid Chromatography (HPLC) Analysis of Juice Samples (mg/g) for the 50°C Runs

L/S Ratio	Stachyose/ Raffinose	Sucrose/ Unknown #1	Glucose	Fructose/ Arabinose	Lactic Acid	Acetic Acid
5.0	1.12 ± 0.11	0.64 ± 0.04	1.10 ± 0.12	4.57 ± 0.17	4.48 ± 0.13	0.58 ± 0.24
4.5	1.02 ± 0.67	0.74 ± 0.36	1.33 ± 0.67	4.81 ± 2.29	4.44 ± 1.92	0.77 ± 0.51
4.0	1.93 ± 0.18	1.21 ± 0.18	2.02 ± 0.18	7.90 ± 0.68	6.90 ± 0.62	1.73 ± 0.18
3.5	1.75 ± 0.49	0.86 ± 0.34	1.22 ± 0.40	6.88 ± 1.39	6.21 ± 1.01	1.57 ± 0.39
2.5	2.40 ± 0.42	1.20 ± 0.32	1.71 ± 0.34	8.81 ± 1.15	7.52 ± 0.84	2.39 ± 0.29

L/S Ratio	Unknown #2	Propionic Acid	Ethanol	Total	Sugars	Organic Acids
5.0	0.23 ± 0.04	0.34 ± 0.04	0.73 ± 0.26	13.79 ± 0.54	7.43 ± 0.33	6.36 ± 0.54
4.5	0.17 ± 0.11	0.32 ± 0.10	0.98 ± 0.60	14.58 ± 7.11	7.90 ± 3.98	6.68 ± 3.16
4.0	0.33 ± 0.08	0.54 ± 0.06	1.87 ± 0.15	24.43 ± 2.09	13.06 ± 1.09	11.37 ± 1.05
3.5	0.25 ± 0.09	0.46 ± 0.14	1.77 ± 0.46	21.07 ± 4.59	10.81 ± 2.58	10.26 ± 2.06
2.5	0.34 ± 0.04	0.60 ± 0.07	1.95 ± 0.38	26.92 ± 3.12	14.12 ± 2.22	12.80 ± 0.91

High Performance Liquid Chromatography (HPLC) Analysis of Juice Samples (mg/ml) for the 70°C Runs*

L/S Ratio	Sample	Stachyose/ Raffinose	Sucrose/ Unknown #1	Glucose	Fructose/ Arabinose	Lactic Acid	Acetic Acid
5.0	6A	0.72	0.49	1.16	4.90	3.88	0.97
	B	0.94	0.58	1.18	5.06	4.02	1.08
	C	1.11	0.79	1.35	5.41	4.34	1.21
		0.92 ± 0.20	0.62 ± 0.15	1.23 ± 0.10	5.12 ± 0.26	4.08 ± 0.24	1.09 ± 0.12
4.0	7A	1.29	0.71	1.52	6.27	5.23	1.46
	B	0.63	0.47	0.98	4.10	3.64	0.42
	C	0.47	0.36	0.77	3.15	2.84	0.31
		0.80 ± 0.43	0.51 ± 0.18	1.09 ± 0.39	4.51 ± 1.60	3.90 ± 1.22	0.73 ± 0.63
3.0	8A	2.31	1.56	2.62	8.74	8.24	2.14
	B	2.54	1.66	2.78	9.52	8.94	2.32
	C	2.52	1.62	2.76	9.49	9.06	2.39
		2.46 ± 0.13	1.61 ± 0.05	2.72 ± 0.09	9.25 ± 0.44	8.75 ± 0.44	2.28 ± 0.13
2.0	9A	3.33	2.07	3.55	12.40	11.68	2.93
	B	3.52	2.16	3.70	13.23	12.63	3.21
	C	3.79	2.32	3.87	14.27	13.46	3.42
	D	3.74	1.82	3.67	14.30	13.86	3.56
		3.60 ± 0.21	2.09 ± 0.21	3.70 ± 0.13	13.55 ± 0.91	12.91 ± 0.96	3.28 ± 0.27
1.5	10A	3.06	1.76	2.76	11.39	10.96	2.80
	B	3.41	1.64	2.86	12.80	12.24	3.28
	C	4.14	2.26	3.43	15.02	13.85	3.72
	D	4.80	2.56	3.70	16.94	15.48	4.18
	E	5.03	2.60	3.76	17.84	16.19	4.26
	F	5.33	2.76	3.81	19.06	17.08	4.42
	G	5.73	2.80	3.84	20.22	17.99	4.68
	H	6.06	2.98	3.86	21.28	18.62	4.93
	I	6.19	2.88	3.72	21.93	19.14	4.84
	J	6.52	3.04	3.04	23.04	19.70	5.14
		5.03 ± 1.18	2.53 ± 0.49	3.48 ± 0.43	17.95 ± 3.92	16.12 ± 2.98	4.22 ± 0.76

L/S Ratio	Sample	Unknown #2	Propionic Acid	Ethanol	Total	Sugars	Organic Acid
5.0	6A	0.00	0.12	0.80	13.04	7.27	5.77
	B	0.00	0.16	0.89	13.91	7.76	6.15
	C	0.07	0.16	0.92	15.36	8.66	6.70
		0.02 ± 0.04	0.15 ± 0.02	0.87 ± 0.06	14.10 ± 1.17	7.89 ± 0.70	6.21 ± 0.47
4.0	7A	0.10	0.23	1.16	17.97	9.79	8.18
	B	0.05	0.10	0.24	10.63	6.18	4.45
	C	0.02	0.10	0.16	8.18	4.75	3.43
		0.06 ± 0.04	0.14 ± 0.08	0.52 ± 0.56	12.26 ± 5.09	6.91 ± 2.60	5.35 ± 2.50
3.0	8A	0.16	0.34	1.64	27.75	15.23	12.52
	B	0.18	0.40	1.78	30.12	16.50	13.62
	C	0.18	0.40	2.04	30.46	16.39	14.07
		0.17 ± 0.01	0.38 ± 0.03	1.82 ± 0.20	29.44 ± 1.48	16.04 ± 0.70	13.40 ± 0.80
2.0	9A	0.24	0.52	2.49	39.21	21.35	17.86
	B	0.35	0.60	2.48	41.88	22.61	19.27
	C	0.27	0.58	2.55	44.53	24.25	20.28
	D	0.36	0.66	2.52	44.49	23.53	20.96
		0.30 ± 0.06	0.59 ± 0.06	2.51 ± 0.03	42.53 ± 2.54	22.94 ± 1.25	19.59 ± 1.35
1.5	10A	0.15	0.42	1.92	35.22	18.97	16.25
	B	0.49	0.70	1.56	38.98	20.71	18.27
	C	0.28	0.66	3.26	46.62	24.85	21.77
	D	0.52	0.90	3.46	52.54	28.00	24.54
	E	0.32	0.76	3.48	54.24	29.23	25.01
	F	0.34	0.80	3.51	57.11	30.96	26.15
	G	0.34	0.80	3.55	59.95	32.59	27.36
	H	0.38	0.91	3.74	62.76	34.18	28.58
	I	0.35	0.90	3.55	63.50	34.72	28.78
	J	0.55	1.09	3.62	65.74	35.64	30.10
		0.37 ± 0.12	0.79 ± 0.18	3.16 ± 0.76	53.65 ±10.45	28.99 ± 5.85	24.66 ± 4.61

* Average of 2 or 3 HPLC analysis readings.

High Performance Liquid Chromatography (HPLC) Analysis of Juice Samples (mg/g) for the 70°C Runs

L/S Ratio	Stachyose/ Raffinose	Sucrose/ Unknown #1	Glucose	Fructose/ Arabinose	Lactic Acid	Acetic Acid
5.0	0.92 ± 0.20	0.62 ± 0.15	1.23 ± 0.10	5.11 ± 0.26	4.07 ± 0.24	1.09 ± 0.12
4.0	0.80 ± 0.43	0.51 ± 0.18	1.08 ± 0.39	4.49 ± 1.59	3.88 ± 1.21	0.73 ± 0.63
3.0	2.43 ± 0.21	1.59 ± 0.05	2.69 ± 0.09	9.15 ± 0.44	8.65 ± 0.44	2.26 ± 0.13
2.0	3.54 ± 0.21	2.06 ± 0.21	3.64 ± 0.13	13.34 ± 0.90	12.71 ± 0.94	3.23 ± 0.26
1.5	4.93 ± 1.16	2.48 ± 0.48	3.41 ± 0.42	17.60 ± 3.84	15.80 ± 2.92	4.14 ± 0.74

L/S Ratio	Unknown #2	Propionic Acid	Ethanol	Total	Sugars	Organic Acids
5.0	0.02 ± 0.04	0.15 ± 0.02	0.87 ± 0.06	14.08 ± 1.17	7.88 ± 0.70	6.20 ± 0.47
4.0	0.06 ± 0.04	0.14 ± 0.08	0.52 ± 0.56	12.21 ± 5.06	6.88 ± 2.59	5.33 ± 2.49
3.0	0.17 ± 0.01	0.38 ± 0.03	1.80 ± 0.20	29.12 ± 1.46	15.86 ± 0.69	13.26 ± 0.75
2.0	0.30 ± 0.06	0.58 ± 0.06	2.47 ± 0.03	41.87 ± 2.50	22.58 ± 1.23	19.29 ± 1.33
1.5	0.36 ± 0.12	0.77 ± 0.18	3.10 ± 0.74	52.59 ± 10.24	28.42 ± 5.74	24.17 ± 4.52

OVERALL JUICE FLOWRATES

Temperature (°C)	Liquid/Solid Ratio	Run	Juice Flowrate (g/min)
50	5.0	1A	107.60
		B	116.00
		C	111.00
		D	119.60
			113.55 ± 5.31
	4.5	2A	111.10
		B	58.25*
		C	93.91
		D	98.26
			101.09 ± 8.94
	4.0	3A	78.65
		B	84.69
		C	85.70
		D	86.10
			83.78 ± 3.47
	3.5	4A	75.19
		B	71.26
		C	64.89
		D	30.85*
			70.45 ± 5.20
	2.5	5A	61.27
		B	41.67
		C	55.17
			52.70 ± 10.03

OVERALL JUICE FLOWRATES cont.

Temperature (°C)	Liquid/Solid Ratio	Run	Juice Flowrate (g/min)
70	5.0	6A	121.12
		B	105.81
		C	115.77
			114.23 ± 7.77
	4.0	7A	84.33
		B	82.10
		C	82.54
			82.99 ± 1.18
	3.0	8A	50.26
		B	46.84
		C	57.52
			51.54 ± 5.45
	2.0	9A	35.62*
		B	11.09*
		C	31.83
		D	30.94
			32.80 ± 2.48
	1.5	10A	15.05
		B	19.20
		C	8.21
		D	16.06
		E	17.55
		F	14.08
		G	8.47
		H	13.07
		I	18.29
		J	13.49
		K	9.87
			13.94 ± 3.82

* Flowrate not used in determining average flowrate, due to clogging of diffusion juice outlet tubing.

OVERALL FLOWRATES

Temperature (°C)	Run	Liquid/Solid Ratio	Wet Ensilage Flowrate (g/min)	Water Flowrate (g/min)	Wet Pulp Flowrate (g/min)	Juice Flowrate (g/min)	Accumulation Term (g/min)
50	1	5.0	26.3 ± 0.1	131	32.24	113.55 ± 5.31	11.51
	2	4.5	26.3 ± 0.1	118	32.90	101.09 ± 8.94	10.31
	3	4.0	26.3 ± 0.1	106	30.81	83.78 ± 3.47	17.71
	4	3.5	26.3 ± 0.1	92	27.26	70.45 ± 5.20	20.29
	5	2.5	26.3 ± 0.1	67	26.08	52.70 ± 10.03	14.52
70	6	5.0	26.3 ± 0.1	131	25.56	114.20 ± 7.77	17.54
	7	4.0	26.3 ± 0.1	104	36.96	82.99 ± 1.18	10.35
	8	3.0	26.3 ± 0.1	78	49.24	51.54 ± 5.45	3.52
	9	2.0	26.3 ± 0.1	53	38.96	32.80 ± 2.48	7.54
	10	1.5	26.3 ± 0.1	39	39.97	13.94 ± 3.82	11.39

Percent Dry Content of Pulp and Ensiled Sweet Sorghum*

Pulp

Temperature (°C)	Day	Liquid/Solid Ratio	Run	Dry Pulp (g) / Wet Pulp (g)
50	1	5.0	1	0.170 ± 0.006
		4.5	2	0.171 ± 0.009
		4.0	3	0.177 ± 0.026
	2	3.5	4	0.172 ± 0.011
		2.5	5	0.155 ± 0.006
70	3	5.0	6	0.139 ± 0.002
		4.0	7	0.165 ± 0.004
		3.0	8	0.164 ± 0.010
		2.0	9	0.153 ± 0.004
	4	1.5	10 A-E	0.162 ± 0.006
		1.5	10 F-J	0.163 ± 0.008

Sweet Sorghum

Day	Silo	Dry Ensilage (g) / Wet Ensilage (g)
8/20	2	0.268 ± 0.003
8/21		0.244 ± 0.002
8/22	1	0.314 ± 0.015
8/23		0.253 ± 0.131

* Average of 3 samples.

Density Measurements of Juice Samples from Diffuser

Temperature (°C)	Liquid/Solid Ratio	Run	Density (g/ml)	No. of Readings
50	5.0	1	1.004 ± 0.007	5
	4.5	2	1.004 ± 0.001	5
	4.0	3	1.009 ± 0.000	5
	3.5	4	1.007 ± 0.000	5
	2.5	5	1.009 ± 0.000	5
70	5.0	6	1.002 ± 0.002	5
	4.0	7	1.005 ± 0.002	5
	3.0	8	1.011 ± 0.001	5
	2.0	9	1.016 ± 0.001	5
	1.5	10 A-E	1.016 ± 0.002	5
	1.5	10 F-J	1.024 ± 0.000	5

Density Measurements of Juice from Sweet Sorghum Ensilage Prepared by the Blender Extraction Method.

Sample	Density (g/ml)	No. of Readings
FE 8/20	1.003 ± 0.0	5
FE 8/21	1.003 ± 0.0	5
FE 8/22	1.003 ± 0.0	5
FE 8/23	1.003 ± 0.0	5

Density Measurements of Juice from the Expression Method for Preparation of Solid Samples for HPLC Anaylsis

Pulp

Temperature (^{o}C)	Liquid/Solid Ratio	Run	Density (g/ml)	No. of Readings
50	5.0	1	1.000 ± 0.000	5
	4.5	2	1.000 ± 0.000	5
	4.0	3	1.000 ± 0.000	5
	3.5	4	0.999 ± 0.001	5
	2.5	5	1.000 ± 0.000	5
70	5.0	6	0.999 ± 0.001	5
	4.0	7	0.999 ± 0.000	5
	3.0	8	0.999 ± 0.000	3
	2.0	9	1.000 ± 0.000	3
	1.5	10 A-E	1.000 ± 0.000	3
	1.5	10 F-J	1.003 ± 0.000	3

Sweet Sorghum

Sample	Density (g/ml)	No. of Readings
FE 8/20	1.043 ± 0.0	3
FE 8/21	1.042*	-
FE 8/22	1.044 ± 0.0	3
FE 8/23	1.039 ± 0.0	3

* Not enough sample for density measurements. Value is average of the other three samples.

Sample Calculations to Convert High Performance Liquid Chromatography (HPLC) Analysis of Sweet Sorghum Ensilage (mg/ml) Prepared by the Blender Extraction Method to Dry Weight (mg/g).

Solve for Dry Weight (mg/g) as Follows:

$$\frac{\text{Component (mg)}}{\text{Solution (mil)}} \times \frac{\text{Solution (ml)}}{\text{Solution (g)}} \times \frac{\text{Solution (g)}}{\text{Wet Ensilage (g)}} \times \frac{\text{Wet Ensilage (g)}}{\text{Dry Ensilage (g)}} = \frac{\text{Component (mg)}}{\text{Dry Ensilage (g)}}$$

Water in Ensilage (g) + Water Added (g) = Solution (g)

$$\left[1 - \frac{\text{Dry Ensilage (g)}}{\text{Wet Ensilage (g)}}\right] \times \text{Wet Ensilage (g) Added to Blender} + \text{Water (g)} = \text{Solution (g)}$$

Substituting in for: Solution (g)

$$\frac{\frac{\text{Component (mg)}}{\text{Solution (ml)}} \times \frac{\text{Solution (ml)}}{\text{Solution (g)}} \times \left[\text{Water (g)} + \left(1 - \frac{\text{Dry Ensilage (g)}}{\text{Wet Ensilage (g)}}\right) \times \text{Wet Ensilage (g)}\right]}{\text{Wet Ensilage (g)} \times \frac{\text{Dry Ensilage (g)}}{\text{Wet Ensilage (g)}}} = \frac{\text{Component (mg)}}{\text{Dry Ensilage (g)}}$$

Sample Calculations cont. -Sweet Sorghum Ensilage from Blender Extraction Method

Expressing Part of the Equation as a Constant:

$$\frac{\frac{\text{Solution (ml)}}{\text{Solution (g)}} \times \left[\text{Water (g)} + \left(1 - \frac{\text{Dry Ensilage (g)}}{\text{Wet Ensilage (g)}}\right) \times \text{Wet Ensilage (g)}\right]}{\text{Wet Ensilage (g)} \times \frac{\text{Dry Ensilage (g)}}{\text{Wet Ensilage (g)}}} = \text{Constant}$$

$$\text{HPLC Analysis} \left[\frac{\text{Component (mg)}}{\text{Solution (ml)}}\right] \times \text{Constant} \left[\frac{\text{Solution (ml)}}{\text{Dry Ensilage (g)}}\right] = \frac{\text{Component (mg)}}{\text{Dry Ensilage (g)}}$$

Constants Derived from Dry Ensilage (g)/Wet Ensilage (g) Ratios, Densities (g/ml), Wet Ensilage (g), and Water (g)

Sample	$\frac{\text{Dry Ensilage (g)}}{\text{Wet Ensilage (g)}}$	$\frac{\text{Solution (g)}}{\text{Solution (ml)}}$	Wet Ensilage (g) Added to Blender	Water (g) Added to Blender	Constant
FE 8/20	0.268	1.003	100.3	779.7	31.6427
FE 8/21	0.244	1.003	100.2	800.0	35.7127
FE 8/22	0.314	1.003	100.3	780.0	26.8706
FE 8/23	0.253	1.003	100.1	786.3	33.8989

Sample Calculations to Convert High Performance Liquid Chromatography (HPLC) Analysis of Sweet Sorghum Ensilage (mg/ml) Prepared by the Expression Method to Dry Weight (mg/g)

Solve for Dry Weight (mg/g) as Follows:

$$\frac{\text{Component (mg)}}{\text{Solution (ml)}} \times \frac{\text{Solution (ml)}}{\text{Solution (g)}} \times \frac{\text{Solution (g)}}{\text{Dry Ensilage (g)}} = \frac{\text{Component (mg)}}{\text{Dry Ensilage (g)}}$$

Solving for the Constant: Solution (ml)/Dry Ensilage (g)

$$\frac{\text{Solution (g)}}{\text{Dry Ensilage (g)}} = \frac{\text{Solution in Ensilage (g)}}{\text{Dry Ensilage (g)}}$$

$$= \frac{\text{Wet Ensilage (g)} - \text{Dry Ensilage (g)}}{\text{Dry Ensilage (g)}}$$

$$= \frac{\frac{\text{Wet Ensilage (g)}}{\text{Wet Ensilage (g)}} - \frac{\text{Dry Ensilage (g)}}{\text{Wet Ensilage (g)}}}{\frac{\text{Dry Ensilage (g)}}{\text{Wet Ensilage (g)}}} = \frac{1 - \frac{\text{Dry Ensilage (g)}}{\text{Wet Ensilage (g)}}}{\frac{\text{Dry Ensilage (g)}}{\text{Wet Ensilage (g)}}}$$

Substituting in for: Solution (g)/Dry Ensilage (g)

$$\frac{\text{Solution (ml)}}{\text{Solution (g)}} \times \frac{1 - \frac{\text{Dry Ensilage (g)}}{\text{Wet Ensilage (g)}}}{\frac{\text{Dry Ensilage (g)}}{\text{Wet Ensilage (g)}}} = \frac{\text{Solution (ml)}}{\text{Dry Ensilage (g)}} = \text{Constant}$$

Sample Calculations cont. -Sweet Sorghum Ensilage from Expression Method

$$\text{HPLC Analysis} \left[\frac{\text{Component (mg)}}{\text{Solution (ml)}}\right] \times \text{Constant} \left[\frac{\text{Solution (ml)}}{\text{Dry Ensilage (g)}}\right] = \frac{\text{Component (mg)}}{\text{Dry Ensilage (g)}}$$

Constants Derived from Dry Ensilage (g)/Wet Ensilage (g) Ratios and Densities (g/ml)

Sample	$\frac{\text{Dry Ensilage (g)}}{\text{Wet Ensilage (g)}}$	$\frac{\text{Solution (g)}}{\text{Solution (ml)}}$	Constant
FE 8/20	0.268	1.043	2.62
FE 8/21	0.244	1.042	2.98
FE 8/22	0.314	1.044	2.09
FE 8/23	0.253	1.039	2.84

Sample Calculations to Convert High Performance Liquid Chromatography (HPLC) Analysis of Pulp (mg/ml) Prepared by the Expression Method to Dry Weight (mg/g)

Solve for Dry Weight (mg/g) as Follows:

$$\frac{\text{Component (mg)}}{\text{Solution (ml)}} \times \frac{\text{Solution (ml)}}{\text{Solution (g)}} = \frac{\text{Component (mg)}}{\text{Solution (g)}}$$

Densities were 1 g/ml for all Expressed Juice Samples; Therefore:

$$\frac{\text{Component (mg)}}{\text{Solution (ml)}} = \frac{\text{Component (mg)}}{\text{Solution (g)}}$$

$$\frac{\text{Component (mg)}}{\text{Solution (g)}} \times \frac{\text{Solution (g)}}{\text{Dry Pulp (g)}} = \frac{\text{Component (mg)}}{\text{Dry Pulp (g)}}$$

Solving for: Solution (g)/Dry Pulp (g)

$$\frac{\text{Solution (g)}}{\text{Dry Pulp (g)}} = \frac{\text{Solution in Pulp (g)}}{\text{Dry Pulp (g)}} = \frac{\text{Wet Pulp (g)} - \text{Dry Pulp (g)}}{\text{Dry Pulp (g)}}$$

$$\frac{\dfrac{\text{Wet Pulp (g)}}{\text{Wet Pulp (g)}} - \dfrac{\text{Dry Pulp (g)}}{\text{Wet Pulp (g)}}}{\dfrac{\text{Dry Pulp (g)}}{\text{Wet Pulp (g)}}} = \frac{1 - \dfrac{\text{Dry Pulp (g)}}{\text{Wet Pulp (g)}}}{\dfrac{\text{Dry Pulp (g)}}{\text{Wet Pulp (g)}}} = \text{Constant}$$

$$\text{HPLC Analysis} \left[\frac{\text{Component (mg)}}{\text{Solution (ml)}}\right] \times \text{Constant} \left[\frac{\text{Solution (ml)}}{\text{Dry Pulp (g)}}\right] = \frac{\text{Component (mg)}}{\text{Dry Pulp (g)}}$$

Sample Calculations cont. - Pulp from Expression Method

Constants Derived from Dry Pulp (g)/Wet Pulp (g) Ratios:

Temperature (^{o}C)	Liquid/Solid Ratio	Dry Pulp (g) / Wet Pulp (g)	Constant
50	5.0	0.170	4.88
	4.5	0.171	4.85
	4.0	0.177	4.65
	3.5	0.172	4.81
	2.5	0.155	5.45
70	5.0	0.139	6.19
	4.0	0.165	5.06
	3.0	0.164	5.10
	2.0	0.153	5.54
	1.5	0.163	5.13

Sample Calculations to Convert High Performance Liquid Chromatography (HPLC) Analysis of Juice Samples (mg/ml) to Juice Concentrations (mg/g)

Solve for Juice Concentrations (mg/g) as Follows:

$$\frac{\text{Component (mg)}}{\text{Juice (ml)}} \times \frac{\text{Juice (ml)}}{\text{Juice (g)}} = \frac{\text{Component (mg)}}{\text{Juice (g)}}$$

$$\text{HPLC Analysis } \left(\frac{\text{Component (mg)}}{\text{Juice (ml)}}\right) \times \frac{1}{\text{Density}} \left(\frac{\text{Juice (ml)}}{\text{Juice (g)}}\right) = \frac{\text{Component (mg)}}{\text{Juice (g)}}$$

Densities Used in Determining Juice Concentrations (mg/g)

Temperature (^{o}C)	Liquid/Solid Ratio	Run	Density (g/ml)
50	5.0	1	1.004
	4.5	2	1.004
	4.0	3	1.009
	3.5	4	1.007
	2.5	5	1.009
70	5.0	6	1.002
	4.0	7	1.005
	3.0	8	1.011
	2.0	9	1.016
	1.5	10	1.020

Sample Calculations for Component Flowrates, Accumulation Terms, and Percent Extraction on Juice and Pulp

Ensilage Component Flowrate:

$$\frac{\text{Wet Ensilage (g)}}{\text{Time (min)}} \times \frac{\text{Dry Ensilage (g)}}{\text{Wet Ensilage (g)}} \times \frac{\text{Component (mg)}}{\text{Dry Ensilage (g)}}$$

$$= \frac{\text{Component (mg)}}{\text{Time (min)}}$$

Pulp Component Flowrate:

$$\frac{\text{Wet Pulp (g)}}{\text{Time (min)}} \times \frac{\text{Dry Pulp (g)}}{\text{Wet Pulp (g)}} \times \frac{\text{Component (mg)}}{\text{Dry Pulp (g)}} = \frac{\text{Component (mg)}}{\text{Time (min)}}$$

Juice Component Flowrate:

$$\frac{\text{Juice (g)}}{\text{Time (min)}} \times \frac{\text{Component (mg)}}{\text{Juice (g)}} = \frac{\text{Component (mg)}}{\text{Time (min)}}$$

Component Accumulation Terms:

Accumulation = Inlet (Ensilage + Water) - Outlet (Pulp + Juice)
(assuming the inlet water concentrations are zero)

$$\text{Component in} \left[\frac{\text{Ensilage (mg)}}{\text{Time (min)}}\right] - \text{Component out} \left[\frac{\text{Pulp (mg)}}{\text{Time (min)}} + \frac{\text{Juice (mg)}}{\text{Time (min)}}\right]$$

$$= \frac{\text{Component Accumulation (mg)}}{\text{Time (min)}}$$

Sample Calculations cont. -Percent Extraction

Percent Extraction on Pulp:

$$\frac{\text{Component Amount in [Ensilage - Pulp]}}{\text{Component Amount in Ensilage}} \times 100\ \% = \%\ \text{Extraction on Pulp}$$

$$\frac{\dfrac{\text{Component in Ensilage (mg)}}{\text{Time (min)}} - \dfrac{\text{Component in Pulp (mg)}}{\text{Time (min)}}}{\dfrac{\text{Component in Ensilage (mg)}}{\text{Time (min)}}} \times 100\ \%$$

$$= \%\ \text{Extraction on Pulp}$$

Percent Extraction on Juice:

$$\frac{\text{Component Amount in Juice}}{\text{Component Amount in Ensilage}} \times 100\ \% = \%\ \text{Extraction on Juice}$$

$$\frac{\dfrac{\text{Component in Juice (mg)}}{\text{Time (min)}}}{\dfrac{\text{Component in Ensilage (mg)}}{\text{Time (min)}}} \times 100\ \% = \%\ \text{Extraction on Juice}$$

APPENDIX B

Time Course for Fermentations

Time vs. Concentration (g/L) for the Clostridium acetobutylicum Fermentation (No. 2) on the 70°C Juice Extract

Time (hr)	Stachyose/ Raffinose	Sucrose/ Unknown #1	Glucose	Fructose	Arabinose	Lactic Acid	Acetic Acid	Unknown #2	Propionic Acid	Ethanol	Butyric Acid	Butanol
0	11.64	3.32	9.55	22.78	2.50	21.86	3.91	0.43	1.11	1.40	0.00	0.47
4	11.99	3.29	9.46	22.48	2.27	21.66	3.96	0.46	1.18	1.62	0.13	0.39
8	12.02	3.36	9.37	22.44	2.27	21.72	3.88	0.41	1.23	1.76	0.10	0.31
16	12.51	3.34	9.28	22.53	2.35	21.83	3.85	0.46	1.15	1.36	0.03	0.35
24	12.28	3.21	9.00	22.68	2.46	21.90	4.03	0.44	1.15	1.72	0.27	0.08
28	12.73	3.42	7.19	22.67	2.26	21.20	4.83	0.50	1.15	1.85	1.27	0.17
30	12.62	3.91	4.28	22.41	2.33	19.82	4.43	0.53	1.23	1.80	2.82	0.25
32	12.73	4.37	0.23	21.82	2.25	17.30	4.69	0.47	1.13	1.60	4.81	0.39
34	12.25	3.02	0.00	19.53	1.45	14.06	4.81	0.46	1.09	1.55	6.29	1.04
36	12.12	2.12	0.00	19.43	1.42	10.58	4.93	0.55	1.24	2.05	7.57	1.50
38	11.22	1.50	0.19	19.85	1.70	7.43	5.39	0.51	1.16	2.76	8.27	1.92
40	11.09	1.79	0.28	19.69	1.64	5.76	5.14	0.46	1.00	1.50	8.36	2.37
42	11.80	2.50	0.52	19.96	1.80	5.54	5.22	0.41	0.99	1.50	8.30	2.70
48	11.75	1.50	0.00	19.70	1.67	5.42	5.79	0.70	1.49	2.57	8.70	2.44
54	11.01	1.90	0.28	20.14	1.79	5.40	5.77	0.83	1.41	2.74	8.90	2.77
60	10.80	1.58	0.00	19.83	1.68	5.34	6.51	0.00	1.87	2.34	8.65	3.90
72	10.42	1.95	0.62	20.39	1.75	5.42	6.36	0.00	1.58	1.97	8.63	2.55
96	10.08	1.91	0.94	20.35	1.79	5.46	6.46	0.22	1.62	2.17	8.55	2.73

Time vs. Concentration (g/L) for the Saccharomyces uvarum Fermentation (No. 1) on the 70°C Juice Extract

Time (hr)	Stachyose/ Raffinose	Sucrose/ Unknown #1	Glucose	Fructose	Arabinose	Lactic Acid	Acetic Acid	Unknown #2	Propionic Acid	Ethanol
0	17.57	3.99	5.29	24.74	2.41	24.76	3.24	3.68	0.91	1.81
4	16.01	3.80	4.46	22.81	2.53	22.85	3.82	5.24	1.04	4.89
8	17.82	4.85	4.23	24.50	3.11	24.73	4.28	4.24	1.03	2.64
12	16.06	5.13	1.56	22.09	2.84	23.40	3.84	4.05	0.86	3.68
14	14.99	5.00	0.85	20.25	2.69	21.99	4.29	3.74	0.81	3.42
16	16.94	5.65	0.96	21.95	3.33	23.96	4.01	4.44	0.95	4.05
18	15.12	4.57	0.64	18.49	2.04	20.36	3.13	3.20	0.96	3.03
20	19.98	4.33	0.19	23.34	3.07	26.02	3.70	4.26	2.84	8.69
22	20.49	6.52	0.00	23.06	2.68	26.10	4.38	4.63	1.50	8.71
24	20.45	5.88	0.50	23.64	3.34	25.96	4.40	4.83	1.51	8.91
30	20.06	5.33	0.34	23.32	2.83	25.89	4.37	4.37	2.71	9.01
36	20.46	6.74	0.52	23.60	2.93	26.16	4.50	4.60	3.08	10.89
42	20.20	4.84	0.00	23.03	4.96	26.07	4.86	4.47	1.38	8.50
48	19.79	5.37	0.00	22.53	2.81	25.60	4.74	4.98	1.45	8.66
72	18.11	3.81	0.35	23.58	3.42	25.59	4.95	4.45	1.57	6.29
96	18.79	4.29	0.44	22.63	3.46	25.17	10.45	7.76	1.98	9.50

Time vs. Concentration (g/L) for the Clostridium acetobutylicum Fermentation (No. 3) on the 70°C Juice Extract

Time (hr)	Stachyose/ Raffinose	Sucrose/ Unknown #1	Glucose	Fructose/ Arabinose	Lactic Acid	Acetic Acid	Unknown #2	Propionic Acid	Ethanol	Butyric Acid	Butanol
0	13.76	4.69	9.52	27.11	27.11	3.16	1.29	1.57	0.69	0.00	1.46
8	14.13	4.68	9.14	27.03	22.64	3.04	1.45	1.50	0.97	0.32	1.57
16	14.12	4.70	7.79	26.98	21.49	3.57	1.22	1.72	0.99	1.19	1.69
24	14.75	4.59	0.14	25.17	16.18	4.28	1.13	1.83	1.04	5.18	1.81
26	16.31	4.05	0.46	22.70	13.30	3.87	1.24	1.55	1.06	5.72	1.87
28	16.63	4.04	0.47	24.49	13.62	3.77	1.39	1.42	1.04	5.71	1.93
30	16.59	3.98	0.48	24.57	13.69	3.97	1.39	1.55	1.11	5.89	2.00
32	16.50	4.02	0.50	24.58	13.63	4.12	1.46	1.46	1.04	5.81	2.08
36	16.49	4.18	0.51	24.37	13.45	3.84	1.39	1.44	0.99	5.75	2.18
42	17.34	4.23	0.50	24.51	13.70	4.14	1.41	1.53	1.07	5.79	2.31
48	17.21	4.30	0.57	24.48	13.72	4.17	1.44	1.53	1.07	5.83	2.53
60	17.13	4.98	0.71	24.26	13.33	4.25	1.54	1.34	1.17	7.46	2.85
72	16.82	4.08	0.61	24.03	13.21	4.69	1.57	1.38	1.14	8.44	3.28
96	16.95	4.85	0.78	24.59	13.50	4.39	1.62	1.37	1.27	6.66	2.96

Time vs. Concentration (g/L) for the Zymomonas mobilis Fermentation (No. 4) on the 70°C Juice Extract*

Time (hr)	Stachyose/ Raffinose	Sucrose/ Unknown #1	Glucose	Fructose/ Arabinose	Lactic Acid	Acetic Acid	Unknown #2	Propionic Acid	Ethanol	Butyric Acid
0	10.18	1.98	7.64	25.34	22.84	5.02	0.52	1.28	1.52	0.00
8	10.20	2.03	7.56	24.99	22.56	4.70	0.69	1.27	1.08	0.05
16	10.37	2.12	7.52	25.12	22.74	4.65	0.58	1.22	1.45	0.00
24	10.24	1.98	7.56	25.12	22.72	5.12	0.39	1.16	1.87	0.14

* Stopped fermentation at 24 hr due to no change in substrate or product concentrations.

Time vs. Concentration (g/L) for the Zymomonas mobilis Fermentation (No. 5) on the 70°C Juice Extract*

Time (hr)	Stachyose/ Raffinose	Sucrose/ Unknown #1	Glucose	Fructose/ Arabinose	Lactic Acid	Acetic Acid	Unknown #2	Propionic Acid	Ethanol	Butyric Acid
0	21.42	1.83	5.65	18.10	16.68	3.54	0.32	0.98	0.80	0.00
12	21.76	1.81	5.66	18.24	16.70	3.82	0.35	0.72	0.86	0.00
18	22.12	1.86	5.66	18.30	16.71	4.01	0.28	0.87	0.86	0.05
24	21.74	1.88	5.58	18.06	16.62	3.81	0.28	0.91	0.66	0.06
48	22.07	1.78	5.61	18.19	16.65	3.94	0.46	0.65	0.72	0.08
60	21.89	1.82	5.64	18.14	16.70	4.12	0.28	0.88	0.88	0.09

* Stopped fermentation at 60 hr due to no change in substrate or product concentrations.

Time vs. Concentration (g/L) for the Clostridium acetobytylicum Fermentation (No. 6) on the 70°C Juice Extract*

Time (hr)	Stachyose/ Raffinose	Sucrose/ Unknown #1	Glucose	Fructose/ Arabinose	Lactic Acid	Acetic Acid	Unknown #2	Propionic Acid	Ethanol	Butyric Acid	Butanol
0	14.53	2.30	10.27	24.58	22.03	4.96	0.48	1.34	1.46	0.00	0.21
12	15.17	2.36	10.16	24.50	21.90	4.68	0.44	1.34	1.50	0.25	0.18
18	15.72	2.21	9.93	23.98	21.40	4.81	0.41	1.31	1.58	0.16	0.24
24	15.94	2.18	10.04	24.16	21.60	4.86	0.42	1.31	1.56	0.15	0.16
30	15.74	2.18	9.84	23.93	21.32	3.90	0.46	1.34	1.32	0.08	0.26
38	15.82	2.09	9.86	23.92	21.26	3.17	0.57	1.77	1.48	0.16	0.06
48	15.53	2.18	9.86	23.67	21.04	3.13	0.42	1.27	1.60	0.14	0.23
62	16.34	2.33	10.02	24.36	21.51	4.80	0.48	1.27	1.61	0.15	0.26

* Stopped fermentation at 62 hr due to no change in substrate or product concentrations.