The research project deals with the enzymatic hydrolysis of cellulose to glucose in ultrafiltration membrane reactors. The reaction is catalyzed by a multienzymatic complex (endoglucanases, exoglucanases and $\beta$-glucosidase). Most cellulase complexes are deficient in $\beta$-glucosidase; furthermore, the latter enzyme is somewhat labile as regards thermal stability. Therefore, a two step process has been studied in which cellobiose conversion to glucose by $\beta$-glucosidase is performed in a membrane reactor which could make use of a stabilized enzyme. In a first reactor cellulose is converted to soluble fractions by native cellulase complexes.

The optimal operating conditions for both reactors have been separately studied (in terms of thermal stability of the enzymes, optimal pH, effects of product inhibition and optimal reaction temperature). As far as the first reactor is concerned, the effect of several chemical and physical pretreatments on the rate of saccharification has been investigated. For the final reaction stage, the possible stabilization of $\beta$-glucosidase by soluble cellulose has been studied together with the effect of high molecular weight substrates accumulation at the membrane surface.
SECTION 1 -
EXTRACTION AND ENZYMATIC HYDROLYSIS OF NATIVE MIXED CELLULOSIC MATERIALS

1.1 Introduction

Most agricultural cellulosic wastes contain three major components, cellulose, hemicellulose and lignin in the ratios of roughly 4:3:3 (1), therefore they are interesting from an energetic point of view. In order to convert the cellulose into glucose and then, via fermentative routes, into ethanol or single cell protein, it is necessary to improve its accessibility to cellulase enzymes, destroying the lignin barrier surrounding cellulose fibers and to reduce the crystalline cellulose into amorphous form (2). In this research project is studied the effectiveness of different chemical and physical pretreatments and the enzymatic hydrolysis of cellulose. The saccharification of cellulose follows this schematic reaction pattern:

\[
\text{Cellulose} \xrightarrow{C_1 + C_X} \text{Oligosaccharides} \xrightarrow{\beta-\text{Glucosidase}} \text{Glucose}
\]

where \(C_x\) and \(C_1\) are Endo and Exoglucanase respectively (3). Being the activity of \(C_1\) and \(C_X\) strongly inhibited by cellobiose and that of \(\beta\)-glucosidase by glucose (4), it is important to continuously remove products of reactions from the reaction volume. In our study the enzymatic saccharification is performed in a membrane reactor. A membrane is selected which completely retains the enzymes and the macromolecular substrates, while the low molecular weight products are removed from the reaction volume. In such a way the product inhibition is controlled and a complete hydrolysis of the cellulosic substrate is possible.

This report, at the end of first year of the research program, is mainly concerning with the discussion of the following items:

i- optimal reaction conditions for the conversion of cellulose into oligosaccharides in a flat membrane reactor (of the stirred type)

ii- method for the determination of thermal stability of the different enzymes present in the cellulase complex

iii- effect of pretreatment on the enzymatic saccharification of olive wastes.

The present study fully covers points 1.1 and 1.2 of the proposal and partially points 2.2 and 4.

1.2 Results and discussion

All the experiments have been performed in a flat membrane reactor: volume 70 ml, membrane surface area 14.5 cm\(^2\), membrane Amicon P.M. 10 type molecular weight cut-off 10,000. A detailed description of the apparatus used in this work is reported in (5). The hydrolysis of either commercial cellulosics (Avicel, microcrystalline cellulose from Machery Nagel and CMC from Schuchardt) or native cellulose (olive-oil wastes, "sanse") has been carried out making use of Cellulase from Trichoderma viride (BDH, 0.02 EU per mg.).

The data have been worked out in terms of concentration of reducing groups (R.G.) and glucose (G) in the effluent cell stream. In Fig.1 are reported characteristic curves of \(C_{\text{R.G.}}\) and \(C_G\) as function of process ti-