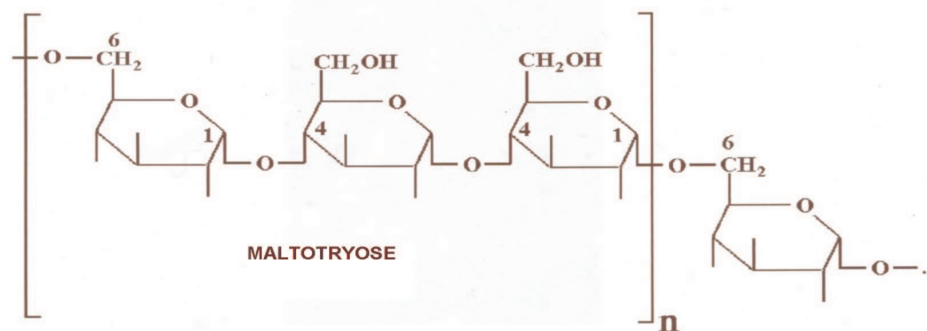


# Pullulan polysaccharide and its derivatives as bioplastic

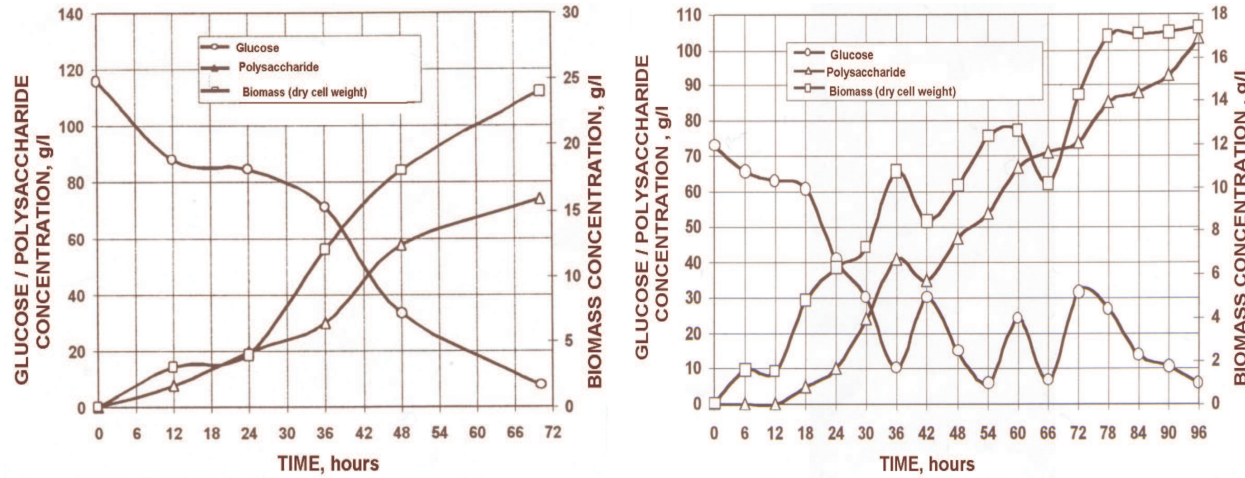


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# Bioreactor fermentation



Typical fermentation profiles of *A. pullulans* ICCF-83 cultivation:  
a) batch; b) fed-batch.

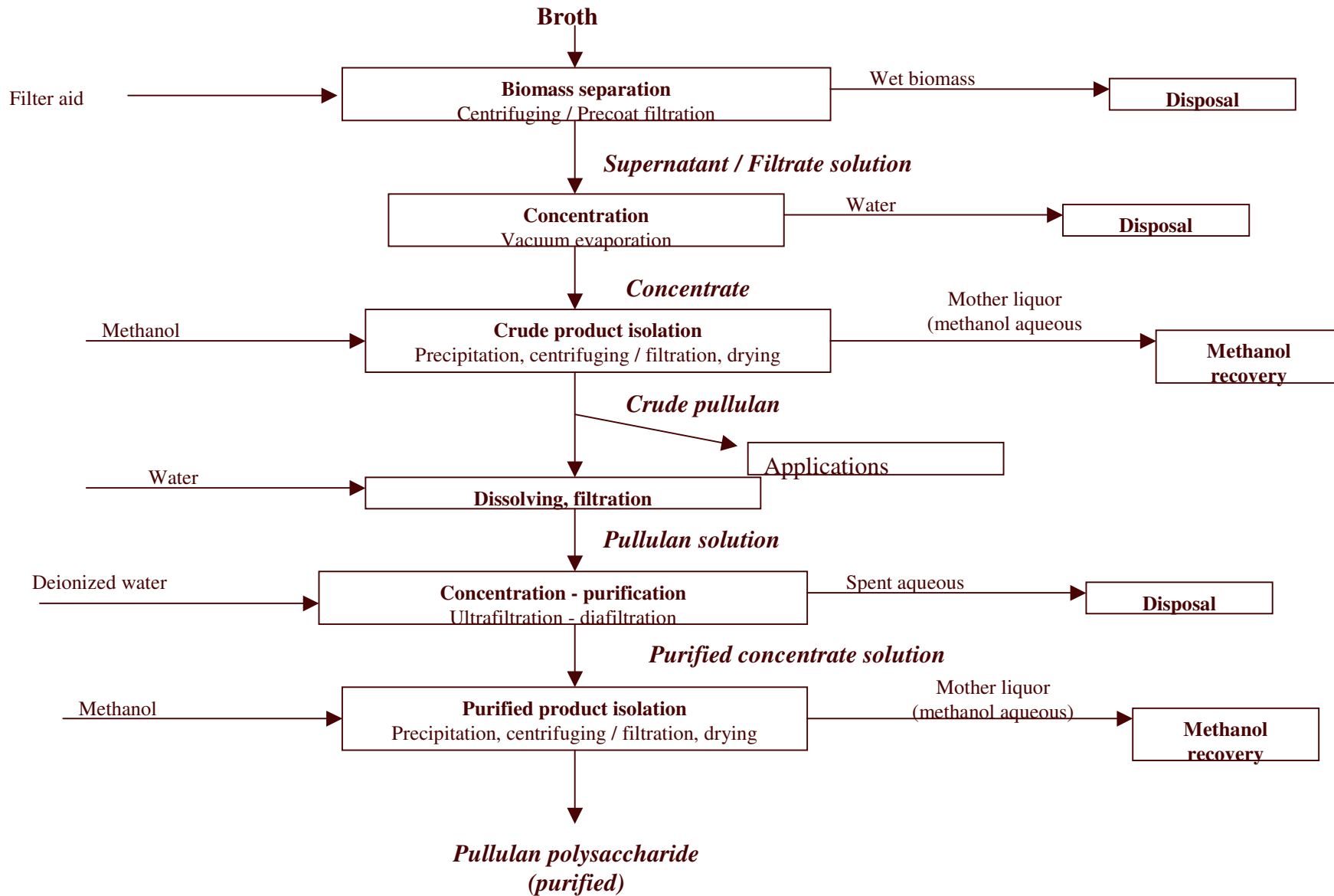
**Table 1:** Pullulan biosynthesis by batch and fed-batch fermentation with *A. pullulans* ICCF-83 (best results) [6].

Bioreactor Cultivation technique	Microfermenter (14 l)		Pilot tank reactor (150 l)	
	batch	fed-batch	batch	fed-batch
<b>Performances:</b>				
<u>Polysaccharide production</u>				
Maximum final concentration, g/l	50 - 74*	103	60	101
Productivity, volumetric g/l/h	1.06	0.99	0.79	0.90
<u>Substrate (carbohydrate)</u>				
<u>conversion yield, %</u>	64	76	66	63

\* On an optimized medium composition

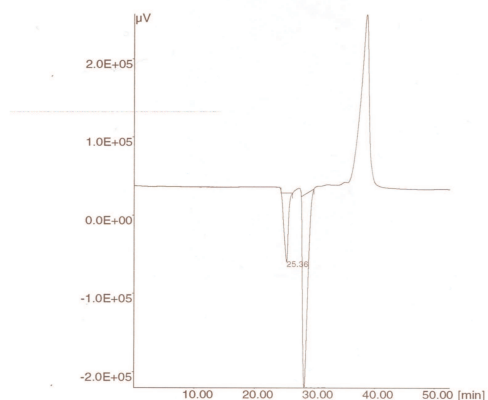
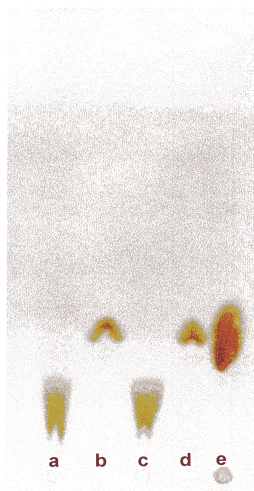
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## Downstream process flowsheet for isolation and purification of pullulan - polysaccharide.

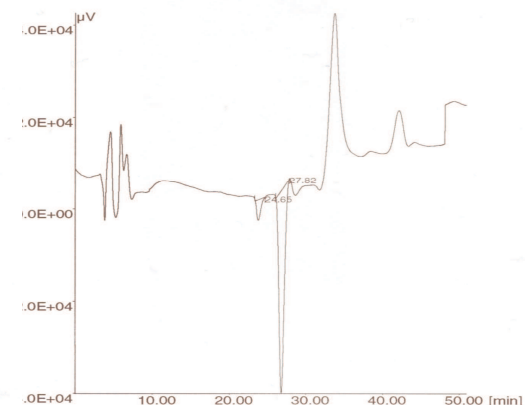


# Product Quality Analysis

Chromatographic tests concerning the purity of pullulan:



(a)



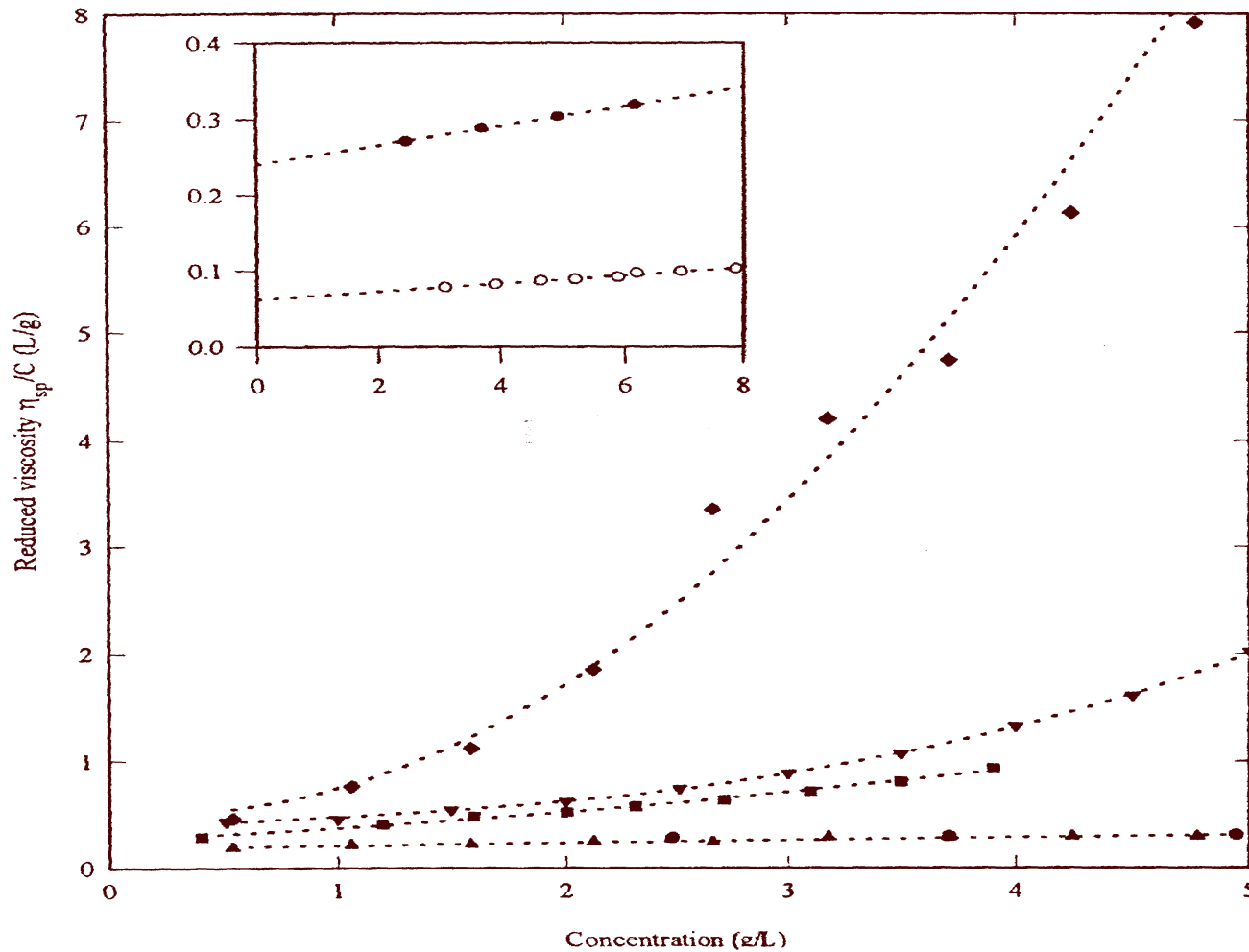
(b)

**TLC:** a, b - reference;  
c, d - ICCF sample;  
a, c - enzymatic hydrolysis (pullulanase);  
b, d - chemical hydrolysis; e - glucose.

**HPLC:** (a) - reference; (b)- ICCF sample

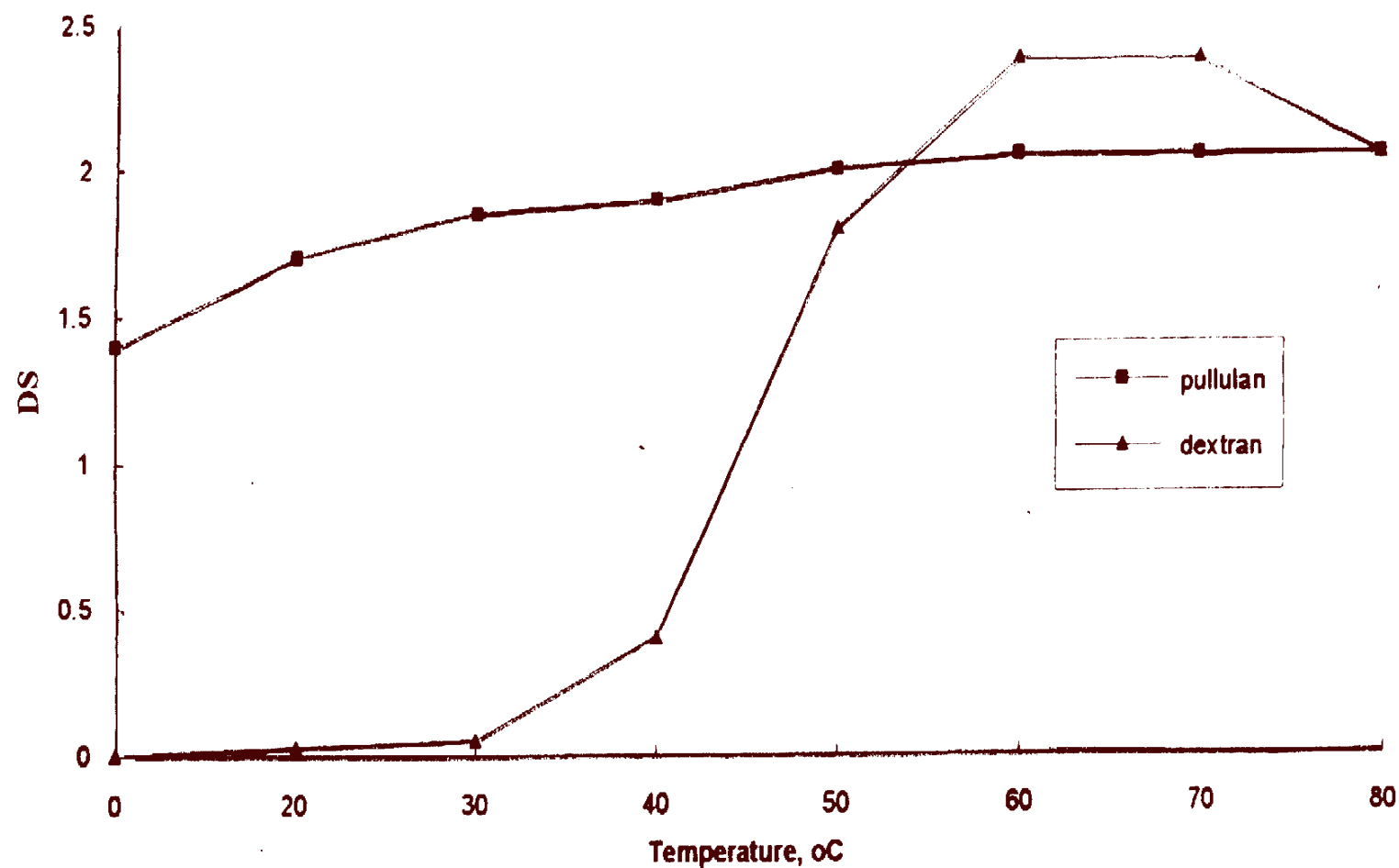
**Reduced viscosity versus polymer concentration for pullulan, carboxymethyl pullulan (CMP) and its associative derivatives, substituted with different content of hydrophobic groups**

(o): pullulan; (●) CMP; (▲) CMP-1.3 C<sub>16</sub>; (■) CMP-3.4 C<sub>16</sub>; (▼) CMP-4.5 C<sub>16</sub>; (◆) CMP-6.8 C<sub>16</sub>.



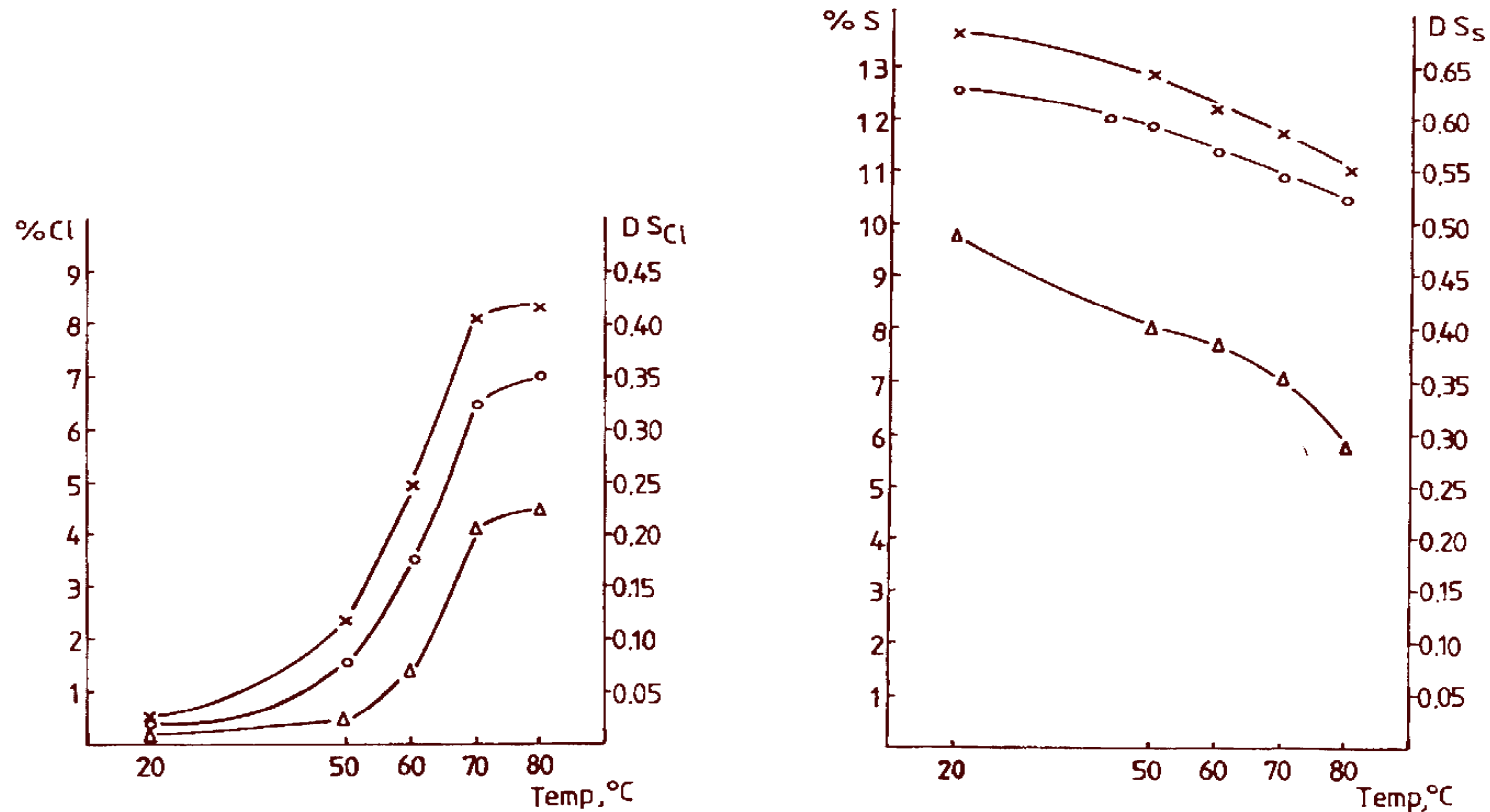
## Effect of the reaction temperature on the DS with chloroacetyl groups of dextran and pullulan

molar ratio chloroacetyl chloride/ glucopyranosic unit: 5/ 1; 6h, solvent: dimethyl formamide.

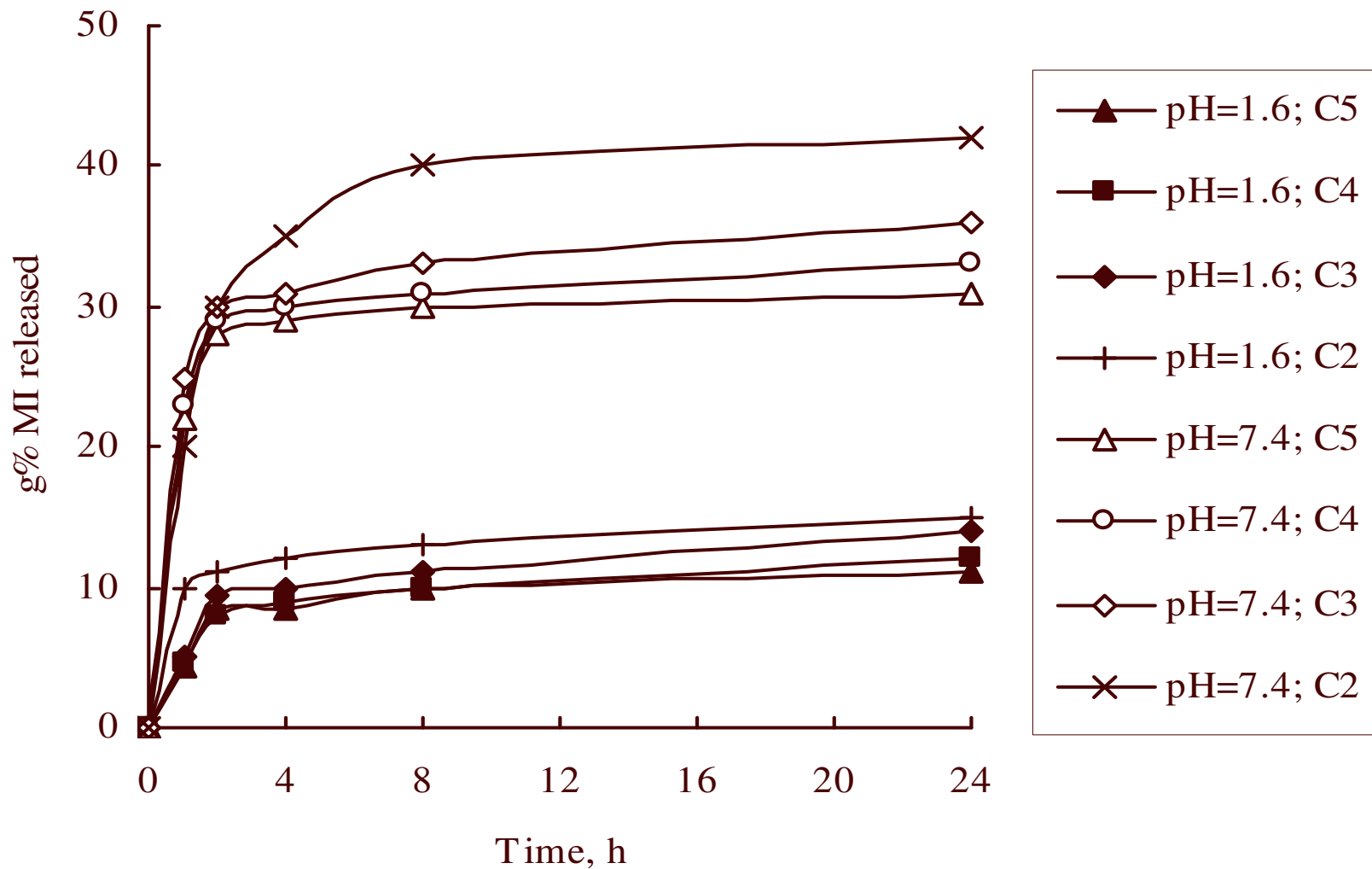


## Influence of the temperature on the %Cl (DSCl) and %S (DSS) of the reaction products;

solvent: pyridine 6 h, molar ratios MsCl/glucosidic unit: ( $\Delta$ ) 1/1; (o) 2/ 1; (x) 3/ 1.



***In vitro* release curves of metronidazole from its conjugates with pullulan previously chloroacylated with various alkyl length**



# Conclusions

- **Mutant high-producing strains of *Aureobasidium pullulans***
- **Biosynthetic performances of pullulan polysaccharide fermentation:**
  - final polysaccharide concentration (g/l):
    - batch: 50 - 74
    - fed-batch: 101 - 103
  - volumetric productivities (g/lh):
    - 0.8 - 1.1
  - substrate conversion yield (%):
    - 63 - 76
- **Downstream isolation and purification process - purified, narrow MW distributed product.**
- **Synthesis of new pullulan derivatives:**
  - cross-linked
  - with reactive groups
  - associative (amphiphilic) polymers
- **Good outlook for a further development of:**
  - a commercial process
  - synthesis of new products
  - enlarging the field of potential applications and knowledge