IJISET - International Journal of Innovative Science, Engineering & Technology, Vol. 2 Issue 12, December 2015.
www.ijiset.com
ISSN 2348 – 7968

Optimization of Acid Hydrolysis Process for Free Glucose Recovery From Starch

Ram Chavan [1], Kunjan Saxena [2] and Dhananjay Tigote [3]

[1,] Lovely Professional University, Phagwara, Punjab-144411
rams.ren22@gmail.com,08805919254
[2,] Lovely Professional University, Phagwara, Punjab-144411
[3] SRTMU, Nanded, Maharashtra-431606

Abstract

In this study experiments were carried out to optimize the acid hydrolysis process to release the maximum amount of free glucose molecules. Two acids were considered; concentrated HCl and concentrated H_2SO_4.Three approaches were followed; cold acid treatment, hot acid treatment and acid treatment combined with moist heat under pressure. For each hot acid treatment by individual acids three parameters were optimized i.e. acid concentration, heating time and heating temperature. The hot acid treatment found to be efficient than cold acid treatment but acid treatment combined with moist heat under pressure is most efficient among them. Increasing the concentrations of acids does not significantly increase the release of free glucose from starch. Free glucose concentrations were estimated by DNS method.

Keywords: *Acid hydrolysis ,glucose, optimization etc.*

1. Introduction

Starch may be hydrolysed by enzymes and inorganic and organic acids. Hydrolysis of starch to glucose by usual acids must be followed by neutralization and subsequent production of large amount of salts [1].So methods should be devised to get maximum free glucose yield by using less quantity of acids.

Acid hydrolysis is an important chemical modification that can significantly change the structural and functional propertiesof starch without disrupting its granular morphology.A deep understanding of the effect of acid hydrolysis on starch structure and functionality is of great importance for starch scientific research and its industrial applications. During acid hydrolysis, amorphous regions are hydrolyzed preferentially,which enhances the crystallinity and double helical content of acid hydrolyzed starch [2].

Starch is a polymer of glucose and contains amylase and amylopectin as building blocks.To release the free glucose,one has beak open the these building blocks by different approaches.[3].

Glucose is slowly destroyed when heated in neutral solutions and the rate is accelerated by sulphuric acid but to a much greater extent by hydrochloric acid.Sulphuric acid is therefore been chosen as a suitable starch hydrolysing agent [4].

2. Materials and Methods:

2.1 Preparation of Starch stock solution:

The starch stock is prepared with 1gm/L Conc. in sterile distilled water. It is used as a substrate for optimization of acid treatment for starch hydrolysis.

2.2 Acid used:

Hydrochloric acid and sulphuric acid:

2.3 Estimation of Glucose concentration:

The glucose released after acid hydrolysis is estimated by DNS method.

2.4 Methods used:

Here during optimization of acid hydrolysis of Starch following factors are optimized with respect to Glucose yield:

- ❖ Type of acid
- ❖ Concentration of acid
- ❖ Heating time
- ❖ Heating temperature

Total of five different conditions are considered with varying acid concentration 1 to 5 % (V/V) and designated as H1, H2, H3, H4, and H5 for Conc. HCl and S1, S2, S3, S4, S5 for Conc.H_2SO_4 respectively. In Control, no acid is added.

Note:

- Optimization is done by using soluble Starch as a substrate.
- Standard Starch stock is prepared with Conc. Of 1 g/L.

Three different methods are employed for optimization of acid hydrolysis of Starch.

A. Cold acid treatment
B. Hot acid treatment
C. Acid hydrolysis in combination to moist heat under pressure (Autoclave) treatment

2.4.1 Cold acid treatment:

ISSN 2348 – 7968

Starch stock (1 g/L) is prepared. Different acid treatments are given using two different acids and labelled accordingly and incubated at room temperature for 15 minutes. Sugar concentration is determined by DNS method. Blank is set by using distilled water and DNS reagent.

2.4.2 Hot acid treatment:

Starch stock (1 g/L) is prepared. Different acid treatments are given using two different acids and labelled accordingly. Here heating period, heating temperature, acid concentration varies sequentially. Sugar concentration is determined by DNS method. Blank is set by using distilled water and DNS reagent.

Sequence of steps followed during optimization of hot acid treatments:

2.4.2.1 Optimization of Acid concentration:

Starch stocks treated with different acid treatments were heated in boiling water bath for 60 minutes and resulting Glucose concentration is determined by DNS method.

2.4.2.2 Optimization of heating time:

The treatment which yields highest Glucose yield was considered for optimization of heating time. Here, the starch stock treated with optimized acid concentration was heated in a boiling water bath for variable heating period (min) and resulting glucose concentration is determined by DNS method.

2.4.2.3 Optimization of heating temperature:

The starch stocks were treated with optimized acid concentration were heated at variable heating temperature at optimized heating time (minutes).The resultant Glucose concentration is determined by DNS method.

2.4.3 Acid hydrolysis in combination to moist heat under pressure (Autoclave) treatment:

Starch stock (1 g/L) is prepared, treated with acids 1 % (V/V) and autoclaved at 15 psi for 15 minutes. Resultant Glucose concentration is determined by DNS method.

Note:

Acid concentration 1% (V/V) is considered to avoid extensive and un-necessary decrease in pH.

3. Observations:

3.1 Observations for cold acid treatments:

Table 1. Different acid treatments and resultant Glucose concentration (g/L) for cold acid Treatment:

Sr.No.	Conc. Of Acid % (V/V)	Resultant Glucose Conc. (g/L)	
		Treatment with HCl	Treatment with H_2SO_4
1	Control(Without acid)	0.3	0.2
2	1	1.01	1.06
3	2	1.04	1.09
4	3	1.08	1.2
5	4	1.2	1.3
6	5	1.3	1.4

3.2 Observation for hot acid treatment:

3.2.1 Observation table for hot acid treatment by using Conc. HCl:

Table2. Optimization of Conc. HCl at 100 ^{0}C at heating for min against different concentrations and resultant Glucose Conc. (g/L):

Sr.No.	Treatment		OD at 550 nm	Glucose Conc. (g/L)
1	Control	**Boiling at 100 ^{0} C for 60 minutes**	0.259	0.5
2	H1		0.887	1.7
3	H2		0.914	1.75
4	H3		0.966	1.85
5	H4		0.992	1.9
6	H5		0.994	1.9

3.2.1.2 Optimization of heating time at H1 treatment and at 100 ^{0}C at different heating time (min) against resultant Glucose yield (g/L)

Table3. Optimization of heating time at H1 treatment and at 100 ^{0}C at different heating time (min) against resultant Glucose yield (g/L):

Sr.No.	Heating time (min)	OD at 550 nm	Glucose Conc. (g/L)
1	0	0.521	1
2	10	0.704	1.35
3	20	0.798	1.53
4	40	0.872	1.67
5	60	0.992	1.9
6	80	0.993	1.9

3.2.1.3 Optimization of heating temperature at H1 treatment and heating time of 60 min at different heating temperatures against resultant Glucose yield (g/L):

Table 4.Optimization of heating temperature at H1 treatment and heating time of 60 min at different heating temperatures against resultant Glucose yield (g/L):

Sr.No.	Temperature ^{0} C	OD at 550 nm	Glucose Conc. (g/L)
1	50	0.714	1.37
2	60	0.756	1.45
3	70	0.83	1.59
4	80	0.84	1.61
5	90	0.95	1.82
6	100	0.997	1.91
7	110	0.998	1.91

3.2.2 Observation table for hot acid treatment by using Conc. H_2SO_4:

3.2.2.1 Optimization of H_2SO_4 Concentration at 100 ^{0}C at heating for 60 min:

Table 5. Optimization of Conc.H_2SO_4 at 100 ^{0}C at heating for 60 min against different concentrations and resultant Glucose Conc. (g/L):

ISSN 2348 – 7968

Sr.No.	Treatment		OD at 550 nm	Glucose Conc. (g/L)
1	Control (without acid)	Boiling at 1oo ° C for 60 minutes	0.311	0.6
2	S1		0.898	1.72
3	S2		0.94	1.8
4	S3		0.982	1.88
5	S4		0.987	1.89
6	S5		0.997	1.91

3.2.2.2 Optimization of heating time (min) at S1:

Table 6. Optimization of heating time at S1 treatment and at 100 ^{0}C against different heating time (min):

Sr.No.	Heating time (min)	OD at 550 nm	Glucose Conc. (g/L)
1	0	0.562	1.08
2	10	0.709	1.36
3	20	0.83	1.59
4	40	0.929	1.78
5	60	0.997	1.91
6	80	0.998	1.91

3.3 Observation for acid hydrolysis in combination to moist heat under pressure (Autoclave) treatment:

Table 7. Resultant glucose yield (g/L) against S1 and H1 after autoclaving:

Sr. NO.	Treatment	OD at 550 nm	Glucose Conc. (g/L)
1	H1	0.217	2.1
2	S1	0.227	2.2

4. Results and Discussions:

4.1 Result of cold acid hydrolysis:

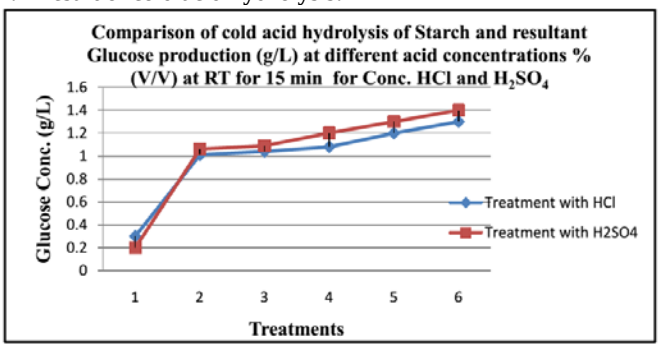

Fig.1 Comparison of cold acid hydrolysis of Starch and resultant Glucose yield (g/L) at different acid concentrations % (V/V) at RT for 15 min for Conc. HCl and H_2SO_4:

4.2 Results for hot acid treatment:

4.2.1 Result for Conc. HCl:

4.2.1.1 Result for optimization of HCl Conc. at 100 ^{0}C at heating for 60 min:

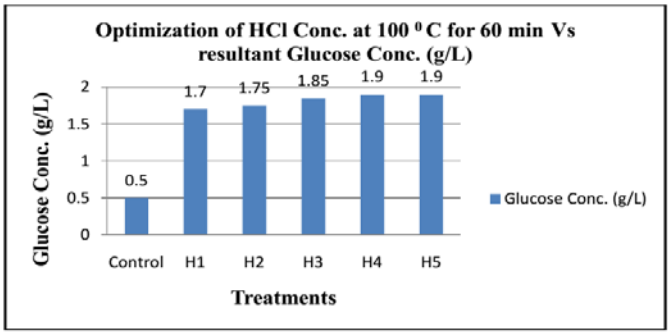

Fig.2 Optimization of Conc. HCl at 100 ^{0}C at heating for 60 min against different concentrations and resultant Glucose Conc. (g/L):

4.2.1.2 Result for optimization of heating time at H1 and at 100 ^{0}C:

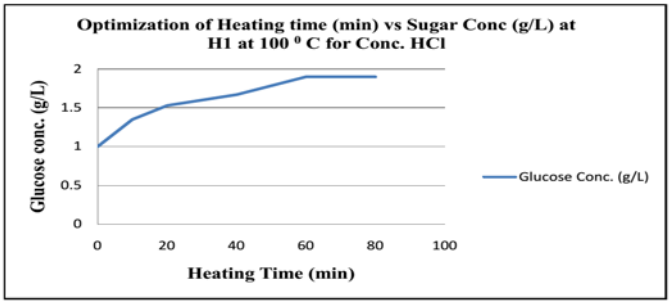

Fig.3 Optimization of heating time at H1 treatment and at 100 ^{0}C at different heating time (min) against resultant Glucose yield (g/L):

4.2.1.3 Result for optimization of heating temperature at H1and heating time of 60 min:

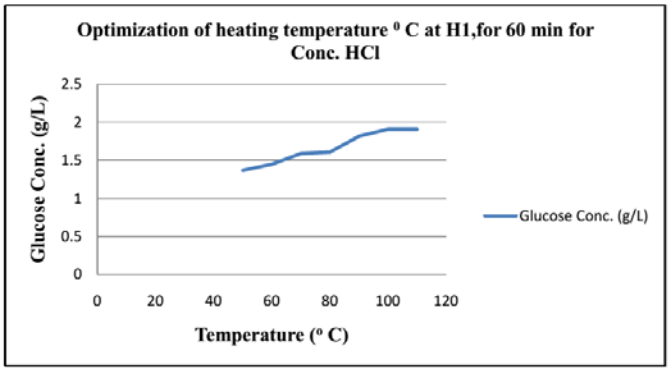

Fig.4 Optimization of heating temperature at H1 treatment and heating time of 60 min at different heating temperatures against resultant Glucose yield (g/L):

4.2.2 Result for Conc. H_2SO_4:

4.2.2.1Result for optimization of H_2SO_4 Concentration at 100 ^{0}C at heating for 60 min:

ISSN 2348 – 7968

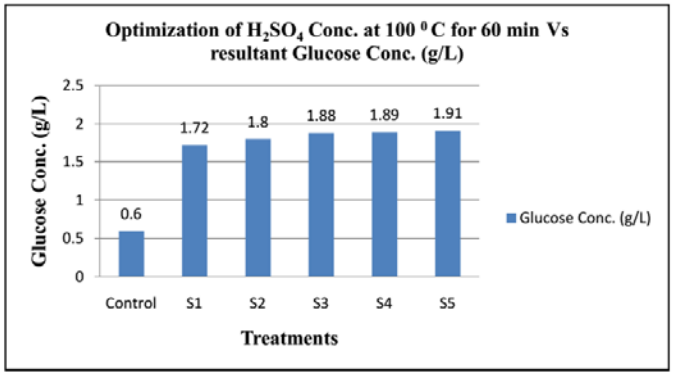

Fig.5 Optimization of Conc. H_2SO_4 at 100 ^{0}C at heating for 60 min against different concentrations and resultant Glucose Conc. (g/L):

4.2.2.1 Result of Optimization of heating time (min) at S1:

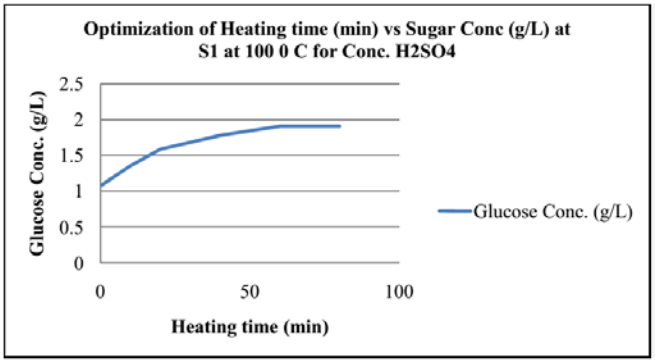

Fig.6 Optimization of heating time at S1 treatment and at 100 ^{0}C at different heating time (min) against resultant Glucose yield (g/L):

4.3 Result for acid hydrolysis in combination to moist heat under pressure (Autoclave) treatment:

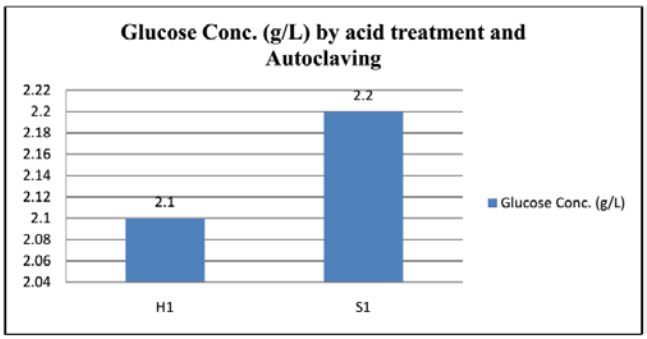

Fig.7 Resultant glucose yield (g/L) against S1 and H1 after autoclaving:

5. Conclusions:

Out of the three acid hydrolysis methods used here i.e. cold acid treatment, hot acid treatment and acid hydrolysis in combination to moist heat under pressure (Autoclave) treatment is more efficient in releasing free glucose from starch. In this method, 1 % (V/V) H_2SO_4 is more efficient than 1% (V/V) of HCl.

References:

[1] A.L. woiciechowski, S.Nitsche, A.Pandey and C.Ricardo, Acid and Enzymatic Hydrolysis to Recover Reducing Sugars from Cassava Bagasse: An Economic Study, Brazilian Archives of Biology and Technology, Vol.45, No.3, 2002, pp.393-400.

[2] S. Wang and L. Copeland, Effect of Acid Hydrolysis on Starch Structure and Functionality: A Review, Critical Reviews in Food Science and Nutrition, Vol.55, No.8, 2013, PP. 1081-1097.

[3] M.HAYEK and R. L. SHRINER, Hydrolysis of Starch by Sulphurous Acid, Industrial and Engineering Chemistry, 1944.

[4] S.J. Pirt and W.J. Whelan, The determination of Starch by Acid Hydrolysis, Journal of the Science of Food and Agriculture,Vol.2,No.5,1951,pp.224-228.

[5] J.H. Choi and S.B. Kim, Effect of Ultrasound on Sulphuric acid Catalysed hydrolysis of Starch, Korean Journal of Chemical Engineering,Vol.11,No.3,1994,pp.178-184.

Ram Chavan: M.Sc. M.Tech in Biotechnology.Currently working as A JRF in BITS Pilani KK Birla Goa Campus on a project entitled “Treatment of Nitrogenous Wastewater t by using microalgae and its utilization for the production of other value added products”Qualified CSIR NET,GATE and ARS Net in Agricultural Biotechology.Published three papers as a main author in international journals.

Kunjan Saxena:M.Tech in Biotechology.Currently working as a Asst.Professor..

Dhananjay Tigote : M.Sc. Zoology