Advanced pilot plant automation for collagen production from hydrobiological waste in Callao, Perú

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***Abstract*– In Peru, about 60% of fish industry raw material ends up as hydrobiological waste, generating spoilage which affects environmental quality. From this waste we can extract collagen by acid-alkalyne process, and using innovative automated processes we can maximize collagen extraction. This article aims to propose a pilot scale plant conceptual process design for collagen extraction from these residues. We used material balance equations in order to scale-up this process and we propose a complete automation using a commercial PLC model. Conceptual process design based on our own experimental phase, as well as batch reactors technical details used in the pilot plant and the justification for the use of these types of reactors and specialized pumps, like sealless centrifugal pump, or stepper motor diaphragm pump, as well as their applications in the collagen extraction process, are explained. Subsequently, the continuous modular design and automatic operation of the equipment is described.**

Keywords — Hydrobiological waste, collagen, automation, PLC, batch reactor, waste reuse.

***Resumen*- En el Perú alrededor del 60% de la materia prima de la industria pesquera termina como residuo hidrobiológico ocasionando el deterioro de la calidad ambiental. De estos desechos podemos extraer colágeno por el tratamiento ácido – base, y mediante innovadores procesos automatizados se maximiza el rendimiento de extracción de colágeno. El presente artículo tiene como objetivo proponer un diseño conceptual para una planta a escala piloto que se emplee para la extracción de colágeno a partir de estos residuos, usando ecuaciones de balance de materiales para escalar este proceso y proponer una automatización completa usando un modelo de PLC comercial. El diseño conceptual del proceso basado en una fase experimental propia, así como el detalle de los reactores tipo batch utilizados en la planta piloto y la justificación del uso de este tipo de reactores y bombas especializadas, como bomba centrífuga sin sello, o bomba de diafragma de motor paso a paso, así como sus aplicaciones en el proceso de extracción de colágeno, son explicados. Posteriormente se describe el diseño modular en línea y funcionamiento automático del equipo.**

Palabras claves— Residuos hidrobiológicos, colágeno, automatización, PLC, reactor discontinuo, reutilización de residuos.

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I. INTRODUCTION

Hydrobiological waste increase is a global problem due to population and industrial growth, generating strong concern among researchers about the use of this waste which may even exceed 60% of the fish industry raw material, generating the opportunity to open up to new little-explored markets in our country to reduce waste production [1]. Based on the Technological Institute of Production, Peru does not register an automated technological plant for fishing industry byproducts use.

Previously, Londoño used PLC Ladder language in the design of his program, modeling the 4 phases of filling, reaction, settlement and decantation in a sequenced batch reactor (SBR), proving the versatility, reliability, and ease to determine the parameters and to operate, allowing its reconfiguration for other processes [2].

An automation system based in PLC programming is an efficient, flexible and adaptable tool for different types of industrial processes since it is versatile to broad range requirements [2]. At present an automated plant efficiently enhances the formation or obtaining of products reducing production time [3].

Giraldo in 2019 studied three fish by-products: red tilapia scales, trout spinal cord and nilotic tilapia skin to identify the best one and standardize the collagen extraction process. They studied the kinetic speed to optimize collagen concentration at T=20°C, Concentration=0.783M. In addition, the alkaline pretreatment and acid extraction were scaled from 200 ml to 2 L with an error of 2.75%, obtaining 84.9% in collagen extraction [4].

Extracted collagen from residues produced in anchovy processing with a 0.1N sodium hydroxide solution, with successive washings to then add a 0.5 M EDTA solution, degreased with 10% butanol and then with 0.5 M acetic acid, resulting in the hydroxyproline content (Hip) in the residues and in the lyophilized collagen, values 6.5 and 52.9 mg of hydroxyproline/g of sample, respectively, concluding that the solubility of lyophilized collagen decreases around 40% at a concentration of 12% NaCl. [5]

This research would have a meaningful impact since it allows to propose an automated design model using cutting edge equipment for collagen extraction [6], thus, the automated

design is a useful tool to evaluate the reuse of waste from the fishing industry in a technical and economic way.

II. MATERIALS AND DEVELOPMENT

2.1. Spectrophotometer UV-visible

The UV VIS spectrophotometer is an analytical equipment that measures the light absorbance of the analyte at different wavelengths to determine its concentration.[7]

2.2 Stepper motor-driven metering pump

Using a stepper motor, the volume dosed is altered by the discharge stroke speed while continuously utilizing 100% of the stroke length, leading to optimum dosing accuracy and better handling of reactant liquids. [8]

2.3. Sealless magnetic pump

Sealless pumps are unique to most process pumps because there is no reliance on a dynamic seal to contain the process fluid. Sealless pumps make use of the process fluid to thin-film lubricate journal type bearings and remove the heat associated with shaft rotation in those bearings.[9]

2.4. Control valve

The control valve manipulates a flowing fluid to compensate for the load disturbance and keeps the regulated process variable as close as possible to the desired set point. The pressure drop of a control valve is directly associated with the energy consumption of the control system [10].

2.5. Double-acting valve actuator

This equipment has air or liquid supplied to both sides of the piston with one side at higher pressure, which achieves the movement required to actuate the valve. Air supplied to one port forces the pistons apart and towards the actuators end caps, with the exhaust air exiting at the other port, a counter-clockwise rotation is achieved [11].

2.6. PLC:

Controller in charge of the automation of the process, allowing the user to define and validate the required variables with the use of a software that was created specifically for the process. A Siemens PLC LOGO 6ED1052-1MD08-0BA0 [12], [13], was used for the automation of the process.

2.7. Food grade materials:

In pipelines and tanks, since we want to make a profit with the produced collagen, we are considering food grade stainless steel, like SS 316. This objective comes from the fact that collagen is widely required by food, pharma and beverage industries. This stainless steel is very resistant to chemical corrosion also. In elastomers, EPDM Sanitary Grade is considered, FDA approved. Finally, in special areas we are considering polypropylene and ETFE-lined equipment, in order to minimize chemical corrosion.

III. PROCEDURE

In the design area, the use of novel instrumentation and new equipment technology has been implemented.

In order to maximize savings, for liquid reactants we are considering the use of stepper motor-driven metering pumps. With this technology, we can dose in different strokes, in both suction and discharge nozzles.

First, we analyzed academic information related to collagen extraction, and we found research from Colombia [14], Malaysia [15] and India [16] where they studied different hydrobiological resources, and they could obtain interesting yields. Based on this information, we started out experimental phase, using the information presented below.

In Fig. 1 the block diagram followed by us, used in the experimental phase and in the conceptual process design is presented.

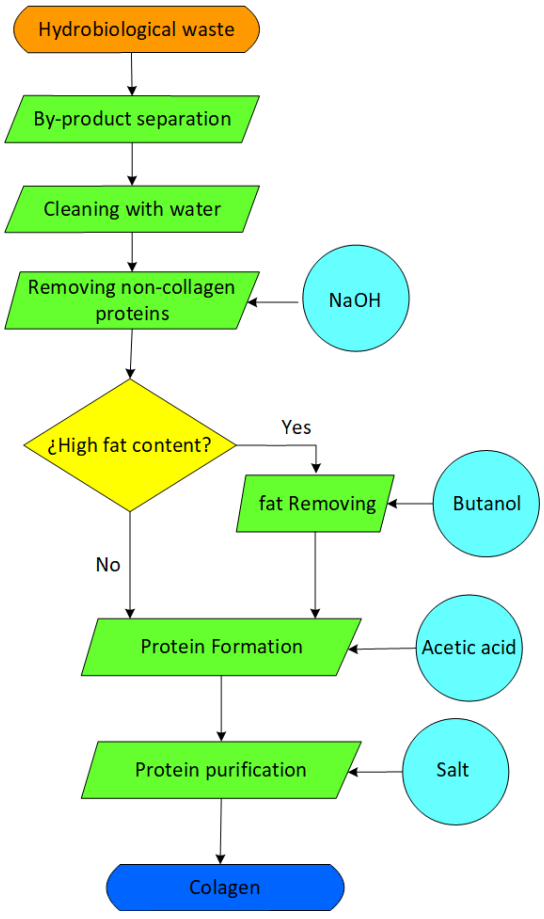


Figure 1
Flow diagram of obtaining collagen

In Fig. 2 shows the sample of hydrobiological residues.

Figure 2
Hydrobiological waste sample

The experimental phase was developed in different stages. First, we disposed the hydrobiological samples in personal coolers with hydrogel and ice, in order to keep a good temperature for it. After it, we segregated the samples, separating the skin from the bones and the blood, washing all the residues three times with distillated water. When we had our samples very clean, we continued with the size reduction. Or target was 1 cm x 1 cm small squares. After it, we weighted the small squares until we had 10 g for each experimental procedure.

When we had all the samples in an appropriated condition, we started with the alkaline treatment, using 1M and 1.2M sodium hydroxide (Merck, pellets, 98%, 40 g/mol) in order to remove fat residues. In some samples, if we saw white fat spots, we used butanol (Spectrum, Reagent, ACS,100%) for a better fat removal. We separate the aqueous phase and the organic phase after it, using a glass separation funnel. The next step was the acid extraction, using 0.6M and 0.8M acetic acid (acetic acid glacial, Mallinckrodt AR,100%) for three hours in order to decompose the proteins from the skin and in that way, we could obtain the collagen. Next, we added 10% m/V sodium chloride, salt (Spectrum, Crystal, Reagent, ACS,5%) for precipitation and sample preservation purposes. The following step consisted on the sample instrumental preparation. For that, we put the samples in small test tubes, and after it we used a laboratory centrifuge. An UV-visible spectrophotometer (Thermo Scientific, AquaMate 8000 UV-VIS) was employed, with 670 nm absorbance wave length. For the calibration curve, using the Bradford 1976 method [17], we constructed it using Bradford's reagent (KMG Chemilab) and Bovine Serum Albumin (BSA) as standard at (0.2, 0.4, 0.6 , 0.8, 1, 1.2, 1.4, 1.6 mg/mL) concentrations of the standard dilution of BSA. (Fig. 3)

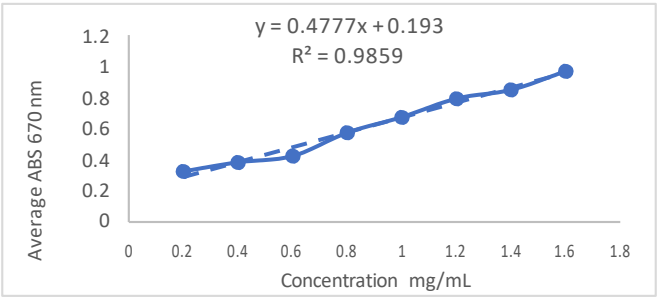


Figure 3
Protein quantification by the Bradford method

For the determination of yields of the experiments at different conditions, the correction of Figure 3 was used. x(Concentration)= ((Y Average Absorbance)-0.193))/0.4777, in order to determine the best conditions for the design of the process proposed where it is shown in Table 4.

Table 4
The absorbance of collagen from hydrobiological waste.

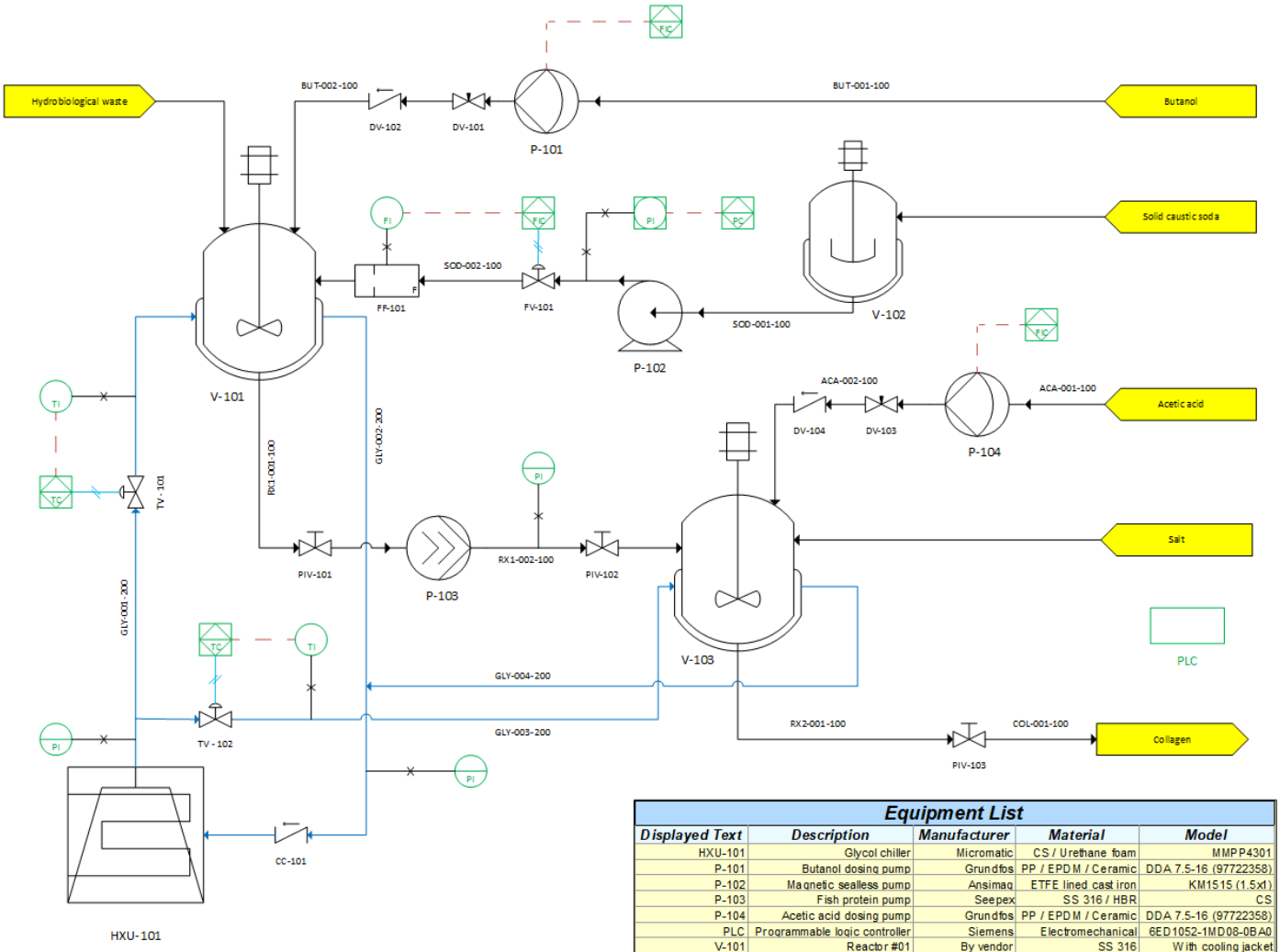
Samples	Absorbance average	Standard Concentration mg/ ml	Protein Concentration mg/ ml	Rendimiento %
NaOH 1M / Acetic acid 0.6 M	0.389	0.41030	4.1030	4.1029
NaOH 1M/ Acetic acid 0.8 M	0.451	0.54009	5.4009	5.4008
NaOH 1.2M/ Acetic acid 0.6 M	0.508	0.65941	6.5941	6.5940
NaOH 1.2M/ Acetic acid 0.8 M	0.665	0.98807	9.8807	9.8806

In concordance with the previous studies in Colombia [14], Malaysia [15], India [16] and our experimental phase, we started the process design stage (conceptual engineering). Based on these yields and results, we developed a conceptual material balance. The material balance results using this technology are shown in the following table 5.

Table 5
Summary of the mass balance

Stage	Chemical reactant	Mass amount	Concentration	Yield	Fluid volume
Input	Hydrobiological	250 g/batch	Pure		
Alkaline treatment	NaOH	100 g	1.5 M	70.32%	0.01 m^3
Fat removal	Butanol	49.05 g	10% m/m	86.11%	
Acid extraction	Acetic acid	21.0074 g	0.7 M	73.98%	0.01222 m^3
Precipitation	Salt	61.1769 g	10% m/V	45.09%	
Output	Collagen	50.5 g	1.08% m/m pp	20.2%	

Based on the kinetic model proposed by Giraldo-Rios, Rios and Zapata-Montoya [14], we propose a scaled-up model. We are considering industrial pilot amounts, taking as reference previous experimental results. With similar stirring speeds in both reactors, and the material balance results, we can continue with the automation of this pilot process plant. The proposed P&ID is shown in the following Fig. 4:



Equipment List

Displayed Text	Description	Manufacturer	Material	Model
HXU-101	Glycol chiller	Micromatic	CS / Urethane foam	MMPP4301
P-101	Butanol dosing pump	Grundfos	PP / EPDM / Ceramic	DDA 7.5-16 (97722358)
P-102	Magnetic sealless pump	Ansimag	ETFE lined cast iron	KM1515 (1.5x1)
P-103	Fish protein pump	Seepex	SS 316 / HBR	CS
P-104	Acetic acid dosing pump	Grundfos	PP / EPDM / Ceramic	DDA 7.5-16 (97722358)
PLC	Programmable logic controller	Siemens	Electromechanical	6ED1052-1MD08-0BA0
V-101	Reactor #01	By vendor	SS 316	With cooling jacket
V-102	Caustic soda preparer	By vendor	PP	With solid mixer
V-103	Reactor #02	By vendor	SS 316	With cooling jacket

Figure 4
P&ID diagram of the pilot plant for the production of collagen from hydrobiological waste

3.1. Descriptions of the figure.

First stage: The process starts with manual cleaning and selection of hydrobiological waste from tilapia. Once the waste was prepared, we continued with the manual disposal in the first reactor V-101.

This reactor is proposed to be built using 316L stainless steel. We are considering 316L SS since is more suitable for food & beverage applications, due to the big chemical resistance, ease to clean (this is very important since its a sanitary process), and welding finish (without any black signal). Also, a cooling jacket is considered, since the adapted kinetic model works in temperatures lower than room temperature. For this cooling application we have chosen glycol.

Second stage: Since the waste will be: skins, bones, scales and fish heads, the alkaline treatment (soda circuit) was set up. This treatment consists of introducing a dilution of soda to the first reactor, V-101. For this, a V-102 mixing tank will be used in which we diluted the solid caustic soda. After that, this liquid stream entered inside a sealless magnetic centrifugal pump P-102.

This technology is considered since liquid caustic soda is susceptible to crystallization, and this process could damage a mechanical seal, located in most of the common centrifugal pumps [14]. In the pump discharge side, we considered a typical pump discharge line arrangement, with a pressure gauge connected to a PLC in the control room as shown in Fig. 5. In the discharge line we considered a flow control loop, formed by a pneumatic control valve controlled by the pressure measured by the orifice plate type flowmeter. The PLC sent the signal for the valve actuator.

Figure 5
PLC SIEMENS - 6ED1052-1MD08-0BA[13]

Third stage: In case the hydrobiological waste contains a considerable fat amount, we considered a fat removing stage. The used solvent is butanol. For its dosing into the system, we considered a stepped motor-driven metering pump P-101. This metering pump was connected to the PLC, and in that way we could control the strokes in the suction and in the discharge side, with different frequencies. Performing this action, we can assure a safe handling of this reagent. The metering line continued into the first reactor V-101.

Fourth stage: After the first reaction (alkaline digestion) this reactor produced a semi solid residue. This stream passed through a pinch valve, located in the discharge tank side. The semisolid stream was pumped by a progressive cavity pump P-103, suitable for handling this kind of media. We considered another pinch valve, for maintenance purposes. At the end of this line, we have the second reactor inlet nozzle.

Fifth stage: In the second batch reactor V-103 the acid digestion starts. We considered a stepped motor-driven metering pump P-104, since the chemical reagent is acetic acid. This second metering pump was connected to the PLC, and in that way we could control the strokes in the suction and in the discharge side, with different frequencies. The discharge line also contains a check valve and a backpressure valve, in order to prevent retro flow and siphoning. This line discharges the fluid into the second reactor V-103 which has a volume of 0.03 m3 as shown in Fig. 6.

Figure 6
Batch reactor

Sixth stage: Once the acid digestion finishes, we continue with the precipitation, using salt. We weighed the salt manually, since this is a batch process.

Seventh stage: When the precipitation process finished, the second reactor V-103 produced a solid moisturized stream. The discharge of the reactor was through another pinch valve. We can proceed with the pure collagen measurement in this product stream.

Eighth stage: As a result of the required temperature is low in both batch reactors, we are considering to use a glycol cooling system. We used a modular chiller for this purpose. The outlet line was connected to the first and second jacket reactor. The glycol outlet line from the rectors returned to the chiller, and in the end of this line we put a check valve, in order to prevent back flow.

IV. Results

In the scale-up process, we have to assure we are meeting the similarities between the lab scale model and the proposed pilot model. According to the mass balance, we calculate the size for each reactor, and in that way, we also can estimate the flow in each pipe. The results are presented in Table 6 and Table 7.

Table 6
Conceptual design for Reactor # 1

Description	*Value*	*Units*	*Criteria*
Fluid total volume	0.1	m^3	
Volume security factor	80	%	
Tank total volume (objective value)	0.125	m^3	
Crown factor (f_{crown})	1		R_{deep} = D
Torus joint constant (k_{torus})	0.06		r_{torus} = 0.06 x D
Diameter (D)	0.530353	m	Goal seek with D value
Deep radius (R_{deep})	0.530353	m	
Torus radius (r_{torus})	0.031821	m	
Crown distance (c)	0.233355	m	c=D/2 - r_{torus}
Extreme height ($h_{extreme}$)	0.089809	m	
Torispherical head volume	0.012083	m^3	
Aspect ratio	1.2		Started in L/D =1.2
Tank total height	0.636424	m	
Cylindrical portion height (h_{cyl})	0.456806	m	
Cylindrical portion volume	0.100914	m^3	
Tank total volume (calculated value)	0.125080	m^3	

Table 7
Conceptual design for Reactor # 2

Description	*Value*	*Units*	*Criteria*
Fluid total volume	0.31	m^3	
Volume security factor	80	%	
Tank total volume (objective value)	0.387125	m^3	
Crown factor (f_{crown})	1		R_{deep} = D
Torus joint constant (k_{torus})	0.06		r_{torus} = 0.06 x D
Diameter (D)	0.7733364	m	Goal seek with D value
Deep radius (R_{deep})	0.7733364	m	
Torus radius (r_{torus})	0.0464002	m	
Crown distance (c)	0.3402680	m	c=D/2 - r_{torus}
Extreme height ($h_{extreme}$)	0.1309549	m	
Torispherical head volume	0.0374615	m^3	
Aspect ratio	1.2000000		Started in L/D =1.2
Tank total height	0.9280037	m	
Cylindrical portion height (h_{cyl})	0.6660938	m	
Cylindrical portion volume	0.3128688	m^3	
Tank total volume (calculated value)	0.3877918	m^3	

Based on material balance, we started with the conceptual process sizing and design. For main pumps, we have sized them using the calculated flowrate. Performance curve for metering in Figure 7, and for sealless magnetic pump we present the performance curve in Figure 8. For main equipment, we propose the following equipment listed in Table 8 and Table 9.

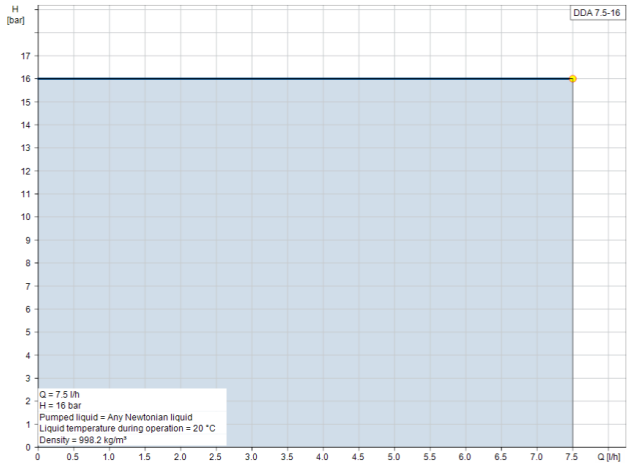


Figure 7
Metering pumps performance curve

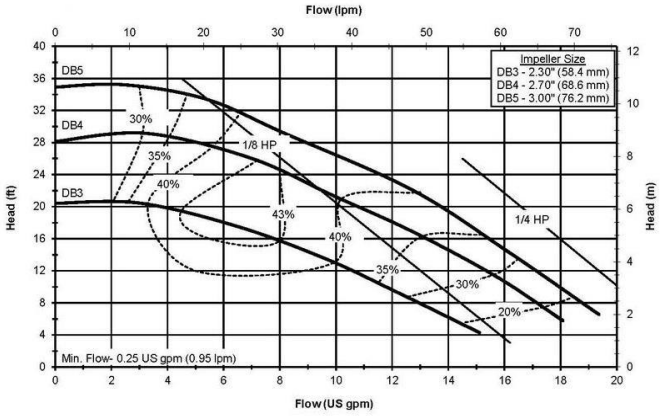


Figure 8
Sealless magnetic pump performance curve

For conceptual equipment sizing and design, as we have described in previous section, we have considered all the equipment showed in Table 8. The pilot plant is going to be controlled using a commercial PLC (listed in Table 8 too). We have considered Siemens PLC due to easy programming steps, and good price – quality ratio. We propose also the use of pinch valves in one stage of the process, since we have a mixed stream (solid + liquid), and for those applications pinch valves are the most suitable vale, due to ease of maintenance and low relative cost. The valve list is shown in Table 7.

Equipment List Description

Displayed Text	*Description*	*Manufacturer*	*Material*	*Model*	*Reference*
HXU-101	Glycol chiller	Micromatic	CS / Urethane foam	MMPP430 DDA 7.5-16 (97722358)	[18]
P-101	Butanol dosing pump	Grundfos	PP / EPDM / Ceramic	DDA 7.5-16	[8]
P-102	Magnetic sealless pump	Ansimag	ETFE lined cast iron	KM1515 (1.5x1)	[19]
P-103	Fish protein pump	Seepex	SS 316 / HBR	CS	[20]
P-104	Acetic acid dosing pump	Grundfos	PP / EPDM / Ceramic	DDA 7.5-16	[8]

				(97722358)	
PLC	Programmable logic controller	Siemens	Electromechanical	6ED1052-1MD08-0BA0	[13]
V-101	Reactor #01	By vendor	SS 316	With cooling jacket	-
V-102	Caustic soda preparer	By vendor	PP	With solid mixer	-
V-103	Reactor #02	By vendor	SS 316	With cooling jacket	-

Table 9
Description of Identification Valve List

Displayed Text	*Description*	*Manufacturer*	*Line Size*	*Valve Type*	*Reference*
PIV-101	Pinch valve	Red valve	0.75 in.	Screw-down	[21]
PIV-102	Pinch valve	Red valve	0.75 in.	Screw-down	[21]
PIV-103	Pinch valve	Red valve	0.75 in.	Screw-down	...[21]

In order to have a better view of our proposed design, an economic detail with individual equipment cost in presented in the Table 10. The prices are considered with the Incoterm CPT Lima (Peru).

Table 10
Equipment Cost List

Displayed Text	*Description*	*Manufacturer*	*Price*
HXU-101	Glycol chiller	Micromatic	USD 4848.07
P-101	Butanol dosing pump	Grundfos	USD 4537.62
P-102	Magnetic sealless pump	Ansimag	USD 6321.50
P-103	Fish protein pump	Seepex	USD 7592.38
P-104	Acetic acid dosing pump	Grundfos	USD 4537.62
PLC	Programmable logic controller	Siemens	USD 258.43
V-101	Reactor #01	By vendor	USD 3500.00
V-102	Caustic soda preparer	By vendor	USD 470.00
V-103	Reactor #02	By vendor	USD 3500.00
PIV-101	Pinch valve	Red valve	USD 739.29
PIV-102	Pinch valve	Red valve	USD 739.29
PIV-102	Pinch valve	Red valve	USD 739.29

V. Some Common Mistakes

Batch reactor process reduce collagen extraction time, since the design allows two batch processes in line, according to material balance. In case it is needed, the first reactor can operate a second basic treatment when the second reactor is working with the acid extraction.

The use of stepper motor technology in metering pumps controlled by a PLC shows chemical reactive savings as is presented in material balance, since the suction and the discharge can be executed in different strokes and frequencies.

Since hydrobiological waste are chemical compounds which are broken down very quickly, it is very important to note that they need to be storaged at a suitable temperature, and in that way a refrigeration system is requiered. Also, it is recommended carry out the analyzes the same day of collection of, in order to obtain the best results.

The proposed design meets sanitary standards, since the materials are suitable for food contact, and in that way the collagen can be used in food, pharma, or beverage industries.

The implementation of this pilot plant can represent a big initial investment, since the technology is modern and advanced, and the materials are expensive. Since the total initial investment cost for this small pilot plant is USD 37783.49, maybe some small companies could not have enough money in order to make this investment magnitude.

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