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Evaluation of the Optimum Conditions for Biotechnological Magnesite Enrichment

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ABSTRACT

Magnesite formulated as MgCO3 is the primary source for magnesium and magnesium derivates. In Turkey, many magnesite deposits cannot be worked out because of high CaCO3 content. Therefore, there are many researchers have applied physical and chemical methods to improve the quality of magnesite. While physical methods are expensive and chemical methods reduce the biological diversity and pollute soil and groundwater, the focus has now been shifted on alternative methods such as biotechnological procedures. For the first time, it was revealed that magnesite enrichment can be carried out biotechnologically by using a nonpathogenic lactic acid bacteria called as Lactococcus sp.(LC) strain. However, the optimum conditions for LC dissolving CaCO3 in magnesite were not determined before. In this research, the optimum conditions for biotechnological magnesite enrichment by LC have been determined with classical methods. To perform optimization experiments, a set of temperature (10-40°C), pH (5-9), particle size (200 mesh-5 mm), solid amount (2-10%), bacterial concentration (1-5%) (~108 cfu/mL) have been tested.

Keywords: *Biotechnological enrichment, Magnesite, Optimization, Lactococcus sp.*

INTRODUCTION

The deposits of ores have been formed at the earth or on earth's surface through different geological processes since the beginning of the earth [1, 2]. Turkey is a rich country in terms of ore variety because of the geological processes it has undergone. The ore variety of Turkey include boron, chrome, iron, copper, manganase, nickel, coal, magnesite, *etc*. Magnesite is the one of the most important ores for economic development of Turkey. Turkey is the second highest magnesite producer in the world and has 205,740 million tons of magnesite reserves that contain %41-48 MgO. Eskişehir, Kütahya, Konya, Çankırı, Erzurum and Erzincan are the provinces having high magnesite reserves in Turkey [3, 4]. Magnesite, the primary source for magnesium and magnesium derivatives, comprises of theoretically 47.6% MgO. However, magnesite ore deposits contain many undesirable impurities such as silisium, iron and calcium. Among all these impurities, expecially high amount of calcium in the form of $CaCO_3$ (≥3%) restrict usability and reduce economic value of magnesite [5]. For industrial applications of magnesite including sinter magnesite that has high flame resistance, magnesite should contain less than 1% calcium carbonate. Since, $CaCO_3$ negatively affects the processing of magnesite through the formation of calcium oxide (CaO) named as 'free form of $CaCO_3$'. Due to water vapor absorbing property of CaO, the stability of the flame resistant material significantly decreases. Besides, in the presence of $CaCO_3$, the stability of flame-resistant materials decreases while the magnesite volume increases [5-7] Up to day, there have been many studies regarding the removal of magnesite impurities and improving magnesite quality by physical and chemical methods. Physical methods such as crushing, scrubbing, and magnetic separation have been used to remove impurities from magnesite. Although physical methods are still widely used, these processes have some disadvantages such as being very expensive, requiring well-designed systems and being insufficient when applied alone [8].

Improving magnesite quality by leaching magnesite with organic or inorganic acids can be defined as 'chemical leaching'. Chemical methods are more effective than physical methods. However, organic and inorganic acids used in leaching processes threaten the environment health through pollution of groundwater and soil [9-13]. Considering the disadvantages of physical and chemical processes, there is a necessity for a new environment friendly, practical and economic process to remove $CaCO_3$. For the purpose of magnesite enrichment, Yanmis et al. [7] have studied the application of bacteria. In their study, they found that *Actinomycetes sp.*, isolated from a cave, significantly lowered the amount of $CaCO_3$ in magnesite, but did not affect the amount of $MgCO_3$ significantly. Then, it has been reported that $CaCO_3$ dissolving *Lactococcus spp.* can remove the clogging of the drip irrigation system pipes [12]. The report of Eroglu et al.2012 has provide the insight to investigate the possibility of magnesite enrichment by this *Lactococcus* (LC) strain [13].

According to our study, in the 5-day batch culture, LC reduced the concentration of $CaCO_3$ of the raw magnesite ore from 2.94 to 1.31% thus, 55.44% enrichment of raw magnesite ore was achieved. Besides, in the 25-day continuous culture, the concentration of $CaCO_3$ of the raw magnesite ore was reduced from 2.94 to 0.57 thus, 80.61% enrichment of raw magnesite ore was achieved. In this study, the optimization experiments for biotechnologically magnesite enrichment using LC have been determined with classical methods. For this, a set of temperature (10-40°C), pH (5-9), particle size (200 mesh-5 mm), solid amount (2-10%), bacterial concentration (1-5%) (~108 cfu/mL) have been tested.

Experimental Procedures

Preparation of Magnesite Samples

The magnesite mineral samples were provided by Turk Mag-Mining Corp in Askale/Erzurum, Turkey. Magnesite samples were crushed, ground, and then sieved by using American Society for Testing and Materials (ASTM) standard. The chemical analyses of the samples were accomplished by using gravimetric and volumetric methods [14]. The analytical result of chemical analyses of magnesite is given in Table 1.

Table1. *The Results of Chemical Analyses of Magnesite*

$CaCO_3$ (%)	2.94
$MgCO_3$ (%)	44.49
LOI (Loss on ignition) (%)	49.11

Acclimation of the Microorganism

The bacterial strain identified as *Lactococcus sp.* was supplied from Yeditepe University Collection (YUC) [9]. To confirm dissolution potential for CaCO3 of the bacterial isolate, Yeast Dextrose CaCO3 (YDC) agar was used (12 g/L yeast extract, 20 g/L dextrose, 5 g/L CaCO3 and 15 g/L agar) (Eroglu et al., 2012; Gulluce et al., 2013). LC was streaked onto YDC and after ten days of incubation at 25 °C, the clear zone around the colonies was observed. The clear zone around the colonies demonstrated that LC had dissolution ability for CaCO3.

Then, the acclimatization process of the LC was performed in 150 mL of the Yeast Dextrose (YD) broth medium (with magnesite ore (12 g/L yeast extract and 20 g/L dextrose) containing 0.2% (w/v) magnesite ore). The bacteria incubated in the described medium at 25C and 150 rpm for 5 days (Yanmis et. al., [13]. Then, 1 mL of the bacteria (10^8cfu/mL) from the acclimation process was inoculated in 250 mL Erlenmeyer containing 150 mL of YD broth medium to determine the optimal enrichment conditions.

Determination of the Optimum Enrichment Conditions

After acclimation process, 1 mL of the bacteria (10^8cfu/mL) was inoculated in 250 mL Erlenmeyer containing 150 mL of YD broth medium to determine the optimal temperature, pH, particle size, solid amount and concentration of bacteria. The reaction contents were stirred at a certain speed for 21 days (504 hours). Dissolution behavior of bacteria for magnesite samples was tested under reaction conditions as follows: temperature from 10 to 40°C, pH from 5 to 9, particle size from 200 mesh to 5 mm, solid amount from 2% to 10%, bacteria amount from %1 to %5 of bacteria with concentration 10^8cfu/mL. Each of the performed experiment was established according to the optimum conditions determined from our previous experimental setup. The experiments of this study have been carried out in three replicates.

Results

According to the experiments of determine optimum leaching temperature, the best results were obtained at 30°C, the results obtained at 20 and 25°C were closed to the results obtained at optimum temperature (Table 2).

While determining the leaching kinetics, another important parameter to be studied is the pH of the medium. The optimal pH range for the growth of LC was 5.0-9.0, thus, these pH values were applied for the effect of pH on dissolution of the $CaCO_3$. The optimal pH for $CaCO_3$ dissolution was determined as pH 6.0 containing 1.16% of solid magnesite. At the same time, the solid magnesite experiments showed that the results of pH 7.0, were closed to the result obtained at optimal pH (pH 6.0). It was observed that pH 8.0 and 9.0 had adverse effect on enrichment process (Table 3).

To determine the effect of different particle size for magnesite enrichment, the experiments were performed for nine particle sizes (200, 140, 60, 35 and 18 mesh and 1, 2, 3 and 5 mm). According to the results obtained, the $CaCO_3$ ratio in solid phase was determined as %1.02 which almost was the desired ratio for 2 mm magnesite sample. Besides, we achieved ideal values of $CaCO_3$ ratio as %0.68 and %0.81 for the particle sizes between 200 mesh and 1 mm, respectively (Table 4).

Table2. *The effect of different temperature on CaO and MgO ion concentration in liquid and $CaCO_3$ and $MgCO_3$ ratio in solid*

Type of medium	Temperature		10	20	25	30	40
Liquid	Control	CaO (%)	0.48	0.52	0.50	0.54	0.52
	LC	CaO (%)	3.02	4.66	5.28	5.45	3.27
	Control	MgO (%)	5.57	5.27	5.31	5.54	5.44
	LC	MgO (%)	15.12	19.32	21.50	21.80	16.53
Solid	Control	$CaCO_3$ (%)	2.53	2.52	2.51	2.56	2.54
	LC	$CaCO_3$ (%)	2.06	1.32	1.12	1.02	1.98
	Control	$MgCO_3$ (%)	44.77	44.78	44.91	44.97	44.95
	LC	$MgCO_3$ (%)	43.76	41.85	41.06	41.25	43.12

Table3. *The effect of different pH on CaO and MgO ion concentration in liquid and on $CaCO_3$ and $MgCO_3$ ratio in solid*

Type of medium	pH		5.0	6.0	7.0	8.0	9.0
Liquid	Control	CaO (%)	0.61	0.58	0.52	0.43	0.31
	LC	CaO (%)	3.99	5.35	4.25	4.15	3.47
	Control	MgO (%)	5.28	5.49	5.36	4.08	2.66
	LC	MgO (%)	20.16	21.93	20.95	20.06	18.85
Solid	Control	$CaCO_3$ (%)	2.38	2.48	2.51	2.65	2.70
	LC	$CaCO_3$ (%)	1.94	1.16	1.48	1.89	2.00
	Control	$MgCO_3$ (%)	44.20	45.10	44.88	46.43	48.12
	LC	$MgCO_3$ (%)	43.16	40.58	41.28	47.89	49.16

Table4. *The effect of different particle size on the CaO and MgO ion concentration in liquid and on $CaCO_3$ and $MgCO_3$ ratio in solid*

	Particle size										
			Millimeter (mm)				Mesh				
Medium Type	Groups	Ions (%)	5	3	2	1	18	35	60	140	200
Liquid	Control	CaO	0.32	0.35	0.35	0.34	0.31	0.31	0.32	0.33	0.35
	LC	CaO	1.06	1.15	1.41	1.05	1.15	1.32	1.28	1.55	1.25
	Control	MgO	2.20	2.40	2.20	2.20	2.30	2.30	2.10	2.40	2.40
	LC	MgO	23.42	23.04	21.42	22.18	23.68	25.46	26.18	26.42	26.28
Solid	Control	$CaCO_3$	2.45	2.42	2.29	2.39	2.19	2.48	2.38	2.24	2.51
	LC	$CaCO_3$	1.65	1.22	1.15	0.95	0.85	0.87	0.86	0.74	0.76
	Control	$MgCO_3$	46.84	46.84	46.84	46.84	46.84	46.84	46.84	46.84	46.84
	LC	$MgCO_3$	44.24	43.95	42.45	41.98	40.16	41.22	40.06	39.85	40.05

The effect of different solid ratio (2, 3, 5, 7,5, 10%) were given in Table 5. According to the results obtained in determining the effect of different magnesite solid ratio, the best results were taken with 2% of magnesite which resulted in % 1.30 $CaCO_3$. On the other hand, 5% of magnesite ratio resulted in % 1.13 $CaCO_3$ which was very close to the optimal magnesite ration (2%) (Table 5).

The effect of bacterial density on magnesite enrichment was another investigated parameter. A variety of bacterial culture ratio (1, 2, 3 and 5%) in a cell density of 10^8cfu/mL was inoculated into the related media and no effect was observed.

Table5. *The effect of different solid ratio on CaO and MgO ion concentration in liquid and on $CaCO_3$ and $MgCO_3$ ratio in solid*

			Solid ratio (%)				
Type of medium			2	3	5	7.5	10
Liquid	Control	CaO (%)	0.48	0.52	0.50	0.54	0.58
	LC	CaO (%)	1.51	1.58	1.62	1.74	1.79
	Control	MgO (%)	5.27	5.33	5.31	5.54	5.44
	LC	MgO (%)	26.20	27.31	27.95	26.48	26.53
Solid	Control	$CaCO_3$ (%)	2.53	2.52	2.58	2.56	2.54
	LC	$CaCO_3$ (%)	1.13	1.15	1.30	1.78	1.94
	Control	$MgCO_3$ (%)	44.77	44.78	44.91	44.97	44.95
	LC	$MgCO_3$ (%)	39.16	39.25	38.50	40.56	40.00

DISCUSSION

The experiments for the determination of optimum leaching temperature were performed at different temperature (10, 20, 25, 30 and 40ºC). Generally, the leaching at high temperature significantly increases the yield of processes. On the other hand, leaching experiments at high temperature result in undesired materials and increase the cost of the processes. In the previous literature regarding the magnesite leaching, the optimum temperature has been determined as 70°C [15, 16]. In our study, it was revealed that LC could survive at a wide range of temperature (10-40°C). The optimum growth temperature for LC was determined as 28°C (data not shown). Even though the best results were obtained at 30°C, the results obtained at 20 and 25°C were closed to the results obtained at optimum temperature. According to the results obtained, it was revealed that the bacteria can survive at 10 and 40°C, but these temperatures were not suitable for the leaching process (Table 2). So we can conclude that the temperature determined for LC to leach the magnesite ore was very favorable for biotechnological applications in term of process cost.

The optimal pH range for the growth of LC was 5.0-9.0, thus, these pH values were applied for the effect of pH on dissolution of the $CaCO_3$. The optimal pH for $CaCO_3$ dissolution was determined as pH 6.0 containing 1.16% of solid magnesite. At the same time, the solid magnesite experiments showed that the results of pH 7.0, were closed to the result obtained at optimal pH (pH 6.0). It was observed that pH 8.0 and 9.0 had adverse effect on enrichment process as these pH values induce $CaCO_3$ precipitating. It is thought that the application of LC in bioleaching process will not produce acidic wastes that can threaten the environmental health. The optimal pH values in the previous magnesite leaching studies were very acidic (pH 2.0-3.0) [17-19]. At this point, the optimal leaching pH of our study seems to be very suitable for environmental health. As known, magnesite mine has the ability to increase the pH of medium because of broken down carbonate compounds. For this reason, inorganic and organic acids have to be continuously added during the magnesite leaching process. The acids used in magnesite leaching process cannot be re-used and are quite expensive. In this case, the acids (inorganic or organic acids) used in the magnesite leaching process bring extra costs [17, 20]. Besides, the wastes with low pH value will decrease the pH of water and land ecosystems and damage the nature seriously. In the long term, this scene will threaten all the life of ecosystems[20]. The bacteria used in our study is a microorganism already isolated from nature, thus, the application of this bacteria will not cause any damage as it was proven to be safe in our previous study [13].

According to the results obtained from experiments to determine optimal particle size, the $CaCO_3$ ratio in solid phase was determined as %1.02 which almost was the desired ratio for 2 mm magnesite sample. Besides, we achieved ideal values of $CaCO_3$ ratio as %0.68 and %0.81 for the particle sizes between 200 mesh and 1 mm, respectively (Table 4). Considering the cost of magnesite enrichment process, 2 mm magnesite sample size will be suitable in order to reduce the relevant cost. In previous studies it has been shown that the magnesite samples in smaller particle size dissolved faster. At this point, our results were supported by the literature [17, 18, 21].

The best results were taken with 2% of magnesite which resulted in % 1.30 $CaCO_3$ through the experiments of the effect of different magnesite solid ratio. On the other hand, 5% of magnesite ratio resulted in % 1.13 $CaCO_3$ which was very close to the optimal magnesite ration (2%). According to the results of the previous studies regarding magnesite leaching kinetics, the leaching efficiency has increased when the amount of the magnesite in the leaching medium was reduced. In this regard, our results about the effect of different solid ratio on leaching efficiency were supported by previous researches [18, 19].

The effect of bacterial density on magnesite enrichment was another investigated parameter. A variety of bacterial culture ratio (1, 2, 3 and 5%) in a cell density of 10^8cfu/mL was inoculated into the related media and no effect was observed.

It is know that the mining industrial organizations produce several million tons of solid inorganic waste materials. It should be taken into account that these waste materials cannot be re-cycled and are estimated to gradually increase. Therefore, new technologies about reuse wastes or reduce of waste amount should be developed for sustainable and cost-effective processing of natural resources. This issue has been highlighted in the 6th European Environmental Impact Plan (EAP). The main strategy of the EAP was to provide 'the sustainable use of natural resources without harming the environment. The strategy of EAP has been strongly supported by European Technology Platform concerning the sustainable use of mineral resources. To control the implementation of strategy aimed to provide the safety of natural resources which is very important for the European economy development, "recycling" is strongly recommended [22].

In this regard, this study provides an alternative in re-cycling magnesite wastes containing high amount of $CaCO_3$. To best of our knowledge, there is no study regarding the optimization of the biotechnological magnesite enrichment. If this enrichment method can improved, it may prevent a possible magnesite crisis in the future and be an environment-friendly method. We believe that the results of this study will be of interest for both mining industry and researchers.

Conclusion

This is the first study determining the optimal conditions for the enrichment of low quality magnesite ore with bacterial applications. The optimal conditions were determined as 140 mesh for particle size, 2% for solid ratio, 30 ºC for reaction temperature, 6.0 for reaction pH. The density of bacteria has no effect on dissolution of carbonates. According to our results, if the application of LC can be developed for industrial processes, it will be useful to reduce the cost of such processes and protect the nature.

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There is no conflict of interest.

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